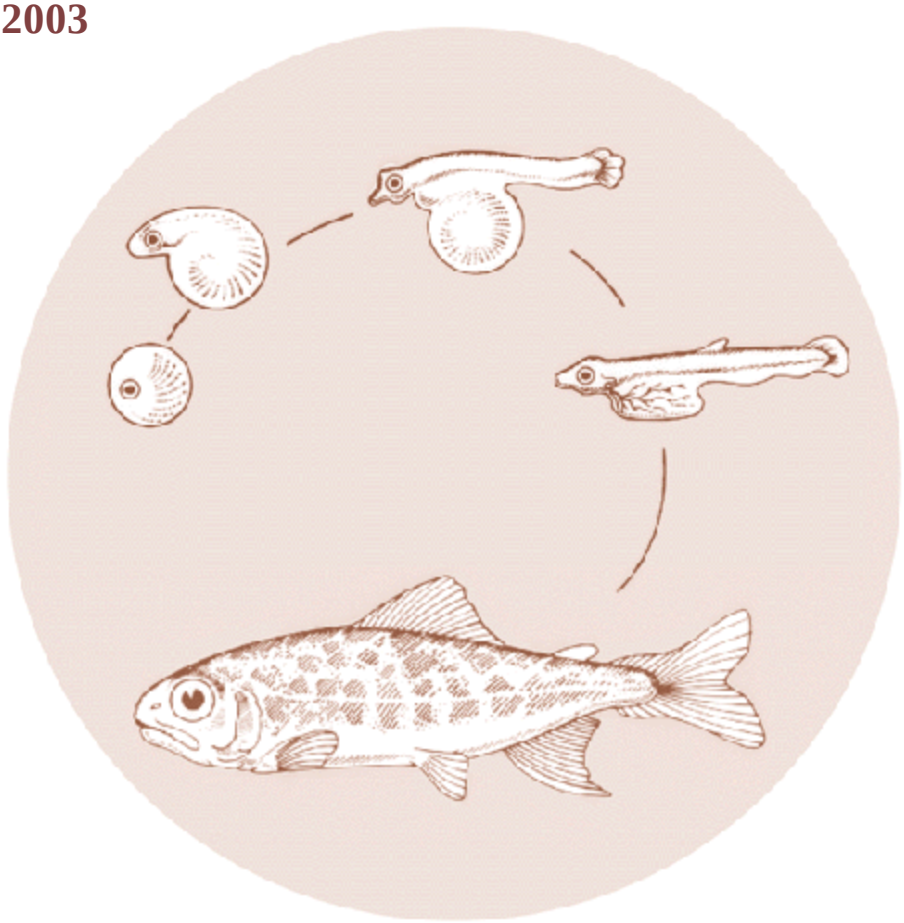
Physiological Assessment of Wild and Hatchery Juvenile Salmonids

Final Report
2003



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PHYSIOLOGICAL ASSESSMENT OF WILD AND HATCHERY JUVENILE SALMONIDS

FINAL REPORT

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Beckman, B. R., D. A. Larsen, C. Sharpe, B. Lee-Pawlak, C. B. Schreck, and W. W. Dickhoff. 2000. Physiological status of naturally-reared juvenile spring chinook salmon in the Yakima River: seasonal dynamics and changes associated with smolting. Transactions of the American Fisheries Society 129, 727-753.

Beckman, B.R., Larsen, D.A, Lee Pawlak, B., and Dickhoff, W.W. (1998). The relationship of fish size and growth to migratory tendencies of Spring chinook salmon (*Oncorhynchus tshawytscha)* smolts. North American Journal of Fisheries Management 18, 537-546.

Larsen, D. A., Beckman, B. R., and Dickhoff, W. W. (2001). The effect of low temperature and fasting during the winter on growth and smoltification of coho salmon. North American Journal of Aquaculture. 63,1-10.

Larsen, D. A., Beckman, B. R., and Dickhoff, W. W. (2001). The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology. 123, 308-323.

EXECUTIVE SUMMARY

It is generally held that hatchery-reared salmonids are of inferior quality and have lower smolt-to-adult survival compared to naturally-reared salmon. The overall objectives of the work performed under this contract were the following:
1) Characterize the physiology and development of naturally rearing juvenile salmonids to:
2) Allow for the design of effective rearing programs for producing wild-like smolts in supplementation and production hatchery programs
3) Examine the relationship between growth rate and size on the physiology and migratory performance of fish reared in hatchery programs.
4) Examine the interaction of rearing temperature and feed rate on the growth and smoltification of salmon for use in producing a more wild-like smolt in hatchery programs.

In Chapter 1 we describe a study examining growth and physiology of two year classes of spring chinook salmon juveniles from the Yakima River, Washington. Fish were sampled from July (3 to 4 months post-emergence) through May (yearling smolt out-migration). Physiological characters measured included liver glycogen, body lipid, gill Na^+-K^+ ATPase, plasma thyroxine, and plasma insulin-like growth factor-I. Distinct physiological changes were found corresponding to season. Summer and fall were characterized by relatively high body lipid and condition factor. Winter was characterized by decreases in body lipid, condition factor, and plasma hormones. An increase in condition factor and body lipid was found in February and March. Finally, April and May were characterized by dramatic changes characteristic of smolting; including, increased gill Na^+-K^+ ATPase activity, plasma T4 and IGF-I and decreased condition factor, body lipid, and liver glycogen. These results create a physiological template for juvenile spring chinook salmon; providing a baseline for comparison with other years, populations, and life history types. In addition, this baseline provides a standard for controlled laboratory experiments and a target for fish culturists who rear juvenile spring chinook salmon for release.

In Chapter 2 we describe experiments that were performed to determine the relative influence of size and growth rate on downstream migratory disposition and physiology in yearling spring chinook salmon (*Oncorhynchus tshawytscha*) smolts. In Part A a group of juvenile chinook salmon was size graded into small and large categories with half the fish in each group reared at an elevated temperature, resulting in four distinct treatment groups: Large Warm (LW), Large Cool (LC), Small Warm (SW), and Small Cool (SC). Fish from warm-water treatment groups displayed significantly higher growth rates than cool-water groups. Fish were tagged and released into a natural creek where downstream movement was monitored. For each of the two releases, fish that migrated past a weir within the first 5 days post-release had significantly higher spring growth rates than fish that did not migrate within that period. Significant differences in length for the same fish were only found in the second release. Also for the second release, fish from the warm water treatment groups were recovered in higher proportions than fish from cool water groups. The results indicate that increased growth

rate in the spring has a positive relation to downstream migratory disposition. Furthermore, there is a relation between smolt size and migration; however, this relation is weaker than that found between growth rate and migration.

In Part B separate groups of juvenile chinook salmon in the four groups (LW, LC, SW, and SC) were sampled for analysis of physiological changes during smoltification. Temporal increases in the growth promoting hormone insulin-like growth factor-I (IGF-I) were found in all groups through the spring. Plasma IGF-I levels were significantly higher in warm-water groups than cool-water groups from late March through May. Size itself appeared to have little relation to plasma IGF-I levels. Simple regression showed a significant relation between plasma IGF-I and growth ($P < .001, R2 = .69$). No differences were found between treatment groups in other physiological parameters assessed (plasma thyroxine, gill Na^+-K^+ATPase, liver glycogen. body lipid). Results suggest that growth rate rather than fish size was a more physiologically relevant aspect of smoltification and subsequent smolt-to-adult survival.

Overall we observed a relatively strong effect of growth rate on downstream migratory tendency, in contrast to its relatively modest effect on physiology. This finding is significant for both the biology of smolt transformation / development and hatchery management. Rapid and directed downstream migration is a most essential element of smoltification. Stimulation of growth of hatchery-reared salmonids during the parr-smolt transformation may improve smolt quality by 1) improving downstream migration and 2) improving seawater tolerance through stimulation of the GH-IGF-I axis. We suggest that hatcheries do not focus on absolute size as a criterion for fish production, but instead develop production schemes which emphasize achieving high rates of fish growth prior to release. However, it is unclear whether simple increases in ration at a constant temperature would also have a stimulatory effect, especially as many hatcheries already feed rations designed to produce optimal growth. A larger-scale test of the relative effects of water temperature and feeding rate and their subsequent effects on growth and smolt performance is warranted.

Finally, based on the dynamic changes in metabolic and endocrine physiology observed in wild salmonids, in Chapter 3 we examine the effect of winter feeding and fasting under both warm (10 ˚C) and cold (2.5 ˚C) water temperature, on growth, smoltification, and metabolic and developmental endocrinology of juvenile coho salmon, *Oncorhynchus kisutch*. Treatments were as follows: Warm-Fed, Warm-Not Fed, Cold-Fed, and Cold-Not Fed during the winter (January - February) prior to smoltification (March-May). All groups were fed and maintained at 10˚C during the smoltification period. In Part A. the following parameters were measured: fork length, weight, condition factor, smolt-associated appearance, whole body lipid levels, and gill Na^+/K^+-ATPase activity. Warm-Fed fish grew continuously throughout the winter and were larger than fish from the other treatments. Fish from the other groups showed little or no growth during January and February. While condition factor decreased significantly in the winter-fasted groups under both warm and cold temperatures, winter whole body lipid levels and smoltification-associated gill Na^+/K^+-ATPase activities were not different between groups.

In Part B the following additional physiological parameters were measured: liver glycogen, hepatosomatic index (HSI), and plasma levels of insulin, insulin-like growth

factor-I (IGF-I), and thyroxine (T4). During the winter liver glycogen, hepatosomatic index, plasma insulin, and IGF-I were highly influenced by manipulations in rearing conditions whereas whole body lipid and plasma T4 were less affected. Plasma insulin levels fluctuated dramatically (from 2 up to 7 ng/ml) in the two cold acclimated groups shortly after the change in temperature. In general, the plasma insulin levels of the Warm-Fed fish were the highest (8-9 ng/ml), the Warm-Not Fed fish the lowest (2-5 ng/ml) and the two cold acclimated groups more variable but intermediate. In contrast, plasma IGF-I levels showed a decline with temperature decrease (from 9 down to 5 ng/ml) and more gradual changes than insulin with the change in feeding. The highest plasma IGF-I levels were found in the Warm-Fed fish (10-15 ng/ml), the lowest in the Cold-Not Fed fish (4-5 ng/ml), and those of the Warm-Not Fed and Cold-Fed fish were intermediate. During the treatment period the T4 levels were relatively unaffected by manipulations in feeding and temperature compared with either insulin or IGF-I.

Taken together data from these two studies suggest that while the insulin, IGF-I, and thyroid axes are differentially regulated under changing seasonal and/or environmental conditions in yearling salmon, winter fasting, even under relatively warm winter water temperatures, may not impair the condition or smoltification of hatchery-cultured salmon. While more studies are warranted, these data suggest that employing a period of fasting during the winter matches the pattern observed in naturally rearing salmonids and does not appear to be detrimental to smolt development.

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CHAPTER 1

"A PHYSIOLOGICAL ASSESSMENT OF WILD JUVENILE SALMON"

Publication Title: Physiological Status of Naturally-Reared Juvenile Spring Chinook Salmon in the Yakima River: Seasonal Dynamics and Changes Associated with Smolting.

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Physiological status of naturally-rearing juvenile spring chinook salmon in the Yakima River: seasonal dynamics and changes associated with smolting.

SUMMARY

Two year classes of spring chinook salmon juveniles from the Yakima River, Washington were sampled from July (3 to 4 months post-emergence) through May (yearling smolt out-migration). Physiological characters measured included liver glycogen, body lipid, gill Na^+-K^+ ATPase, plasma thyroxine, and plasma insulin-like growth factor-I. Distinct physiological changes were found corresponding to season. Summer and fall were characterized by relatively high body lipid and condition factor. Winter was characterized by decreases in body lipid, condition factor, and plasma hormones. An increase in condition factor and body lipid was found in February and March. Finally, April and May were characterized by dramatic changes characteristic of smolting; including, increased gill Na^+-K^+ ATPase activity, plasma T4 and IGF-I and decreased condition factor, body lipid, and liver glycogen. These results create a physiological template for juvenile spring chinook salmon; providing a baseline for comparison with other years, populations, and life history types. In addition, this baseline provides a standard for controlled laboratory experiments and a target for fish culturists who rear juvenile spring chinook salmon for release. The implications of these results for juvenile chinook salmon ecology and life history are discussed.

INTRODUCTION

At an organismal level, one must understand an animals physiological processes if one seeks to understand growth, survival, and reproductive success (Kitchell 1998). Physiological processes in Pacific Salmon have received a great deal of attention (Groot et al. 1995). However, the physiological status of free-living juveniles has been relatively unexplored, previous work has reported on fish reared in either production or experimental hatcheries. Culture conditions may vary from those found in streams and rivers with regard to seasonal temperature profiles, photoperiod, nutrition, and social interactions. These differences might lead to differences in biological characters, resulting in alteration of developmental timing, growth rate, size at age, and body composition. Thus, it may be misleading to apply results from cultured fishes to naturally-rearing juveniles as the physiological status of naturally-rearing fish may not correspond to that of hatchery reared fish.

A better understanding of physiological processes in naturally-rearing juvenile salmon may have two broad implications. First, it will allow better assessment of how environmental variation or perturbation effects individual survival and thus population size. Second, knowledge of these physiological processes may also give us insight into how to more successfully rear salmon in artificial culture. Logic suggests that if we want hatchery fish to match the performance of wild fish, they should resemble wild fish, both physically and physiologically. This is particularly true for chinook salmon (*Oncorhynchus tshawytscha*) supplementation programs, which are receiving wide-spread support and development in the Columbia River Basin (). Supplementation projects are not designed solely to produce fish; rather, the goal of supplementation programs is to increase the size of distinct "wild" populations of salmon, without affecting either their genotypic or phenotypic characteristics. Thus, one might use a physiological template obtained from naturally rearing fish to guide the rearing of fish in supplementation facilities.

Growth and physiological status are associated with the timing and intensity of smolting. Smolting is an important developmental transition in anadromous salmonids that both allows and stimulates juvenile fish to undertake a freshwater to ocean transition. Age, size, and season of smolting varies widely among chinook salmon populations (Healey 1991) and this variation has been attributed to both genetic and environmental factors (Taylor 1990). Examination of smolting in naturally-rearing fish will allow us to better understand this life history variation. In addition, smolting is a critical process for cultured salmon. If salmon are released from culture facilities as smolts (having initiated the smolting process) they are more likely is show a rapid directed migration to the ocean (Zaugg 1981, 1989, Muir 1994b). A rapid migration may both increase survival of juvenile salmon and decrease interactions between cultured fish and wild fish. Thus, the process of smolting in naturally-rearing chinook salmon juveniles is of particular interest.

The objective of this study was to examine the endocrine and physiological status of naturally-rearing spring chinook salmon juveniles in the Yakima River, a medium-sized tributary of the Columbia River in South Central Washington. Yakima River fish belong to a meta-population of spring chinook salmon in the Columbia River that typically smolt as yearling fish in April - May (Meyers et al. 1998). Smolting after a year of freshwater rearing is a common characteristic of "stream-type" chinook salmon of

Alaska, British Columbia, Washington and Oregon (Healey 1991). We began sampling in July, several months post-emergence, and continued sampling on a two to four week interval through smolting in the following spring. In this manner we attempted to construct a thorough seasonal profile of the endocrine and physiological status of juvenile chinook salmon, with special attention paid to the period preceding and including smolting. We report here on two years of study of these fish.

MATERIALS AND METHODS

Study Area

The Yakima River (total length 349 km, drainage area 15,900 km^2) is located in central Washington State, USA (Figure 1). The head waters drain the eastern escarpment of the Cascade Mountain Range (altitude 2,440 m) and flow southeasterly through the Columbia Plateau to its confluence with the Columbia River (altitude 104 m). The Yakima river traverses three distinct river valleys, characterized by irrigated agricultural areas. Important geographical features include the 40 km long Yakima River Canyon (river kilometer (rk) 235), the confluence with the Naches River (drainage area 2,860 km^2) at the town of Yakima (rk 187), and the confluence with the Columbia River near the town of Richland (rk 0). Flow in the Yakima River system is regulated by a series of irrigation reservoirs. Kacheelus, Kachess, and Cle Elum Lakes in the upper Yakima supplement flows from March to August and Bumping and Rimrock Lakes on the Naches River supplement flows during September and October. Temperature and flow data for the Yakima River reported herein were obtained from the U.S Bureau of Reclamation Hydromet data base (USBR PO Box 1749, Yakima WA 98907).

The Yakima Basin contains three closely related, yet distinct, sub-populations of spring chinook salmon: American River, Naches River and tributaries (excluding the American River), and Yakima River Mainstem and its tributaries (Busack and Marshall 1991, Myers et al. 1998). Approximately 60% of adult spawning occurs below the Easton Dam and in the Cle Elum River below Cle Elum Lake in the mainstem Yakima River. The remaining approximately 40% of spawning occurs in the Naches River and its associated tributaries (Fast et al. 1991).

Sampling sites

Our sampling was constrained by seasonal changes in abundance and distribution of chinook salmon as described by Fast et al. (1991). In the spring and summer months the majority of juvenile salmonids in the Yakima Mainstem are distributed above Roza Dam (Figure 2). In the late summer - early fall, seasonally declining temperatures allow fish to occupy the river below the town of Yakima. Finally, in April of their second year, the majority of juveniles outmigrate past Chandler Dam, located in the town of Prosser. Utilizing these general distributional trends, our sampling sites and timing were divided into two broad areas: Above Yakima (June - April): from below Easton Dam (rk 324) to Roza Dam (km 204); and below Yakima (September - May): from the Naches River confluence (km 187) to Zillah (rk 150) (Table 1). In addition, migrant smolts were

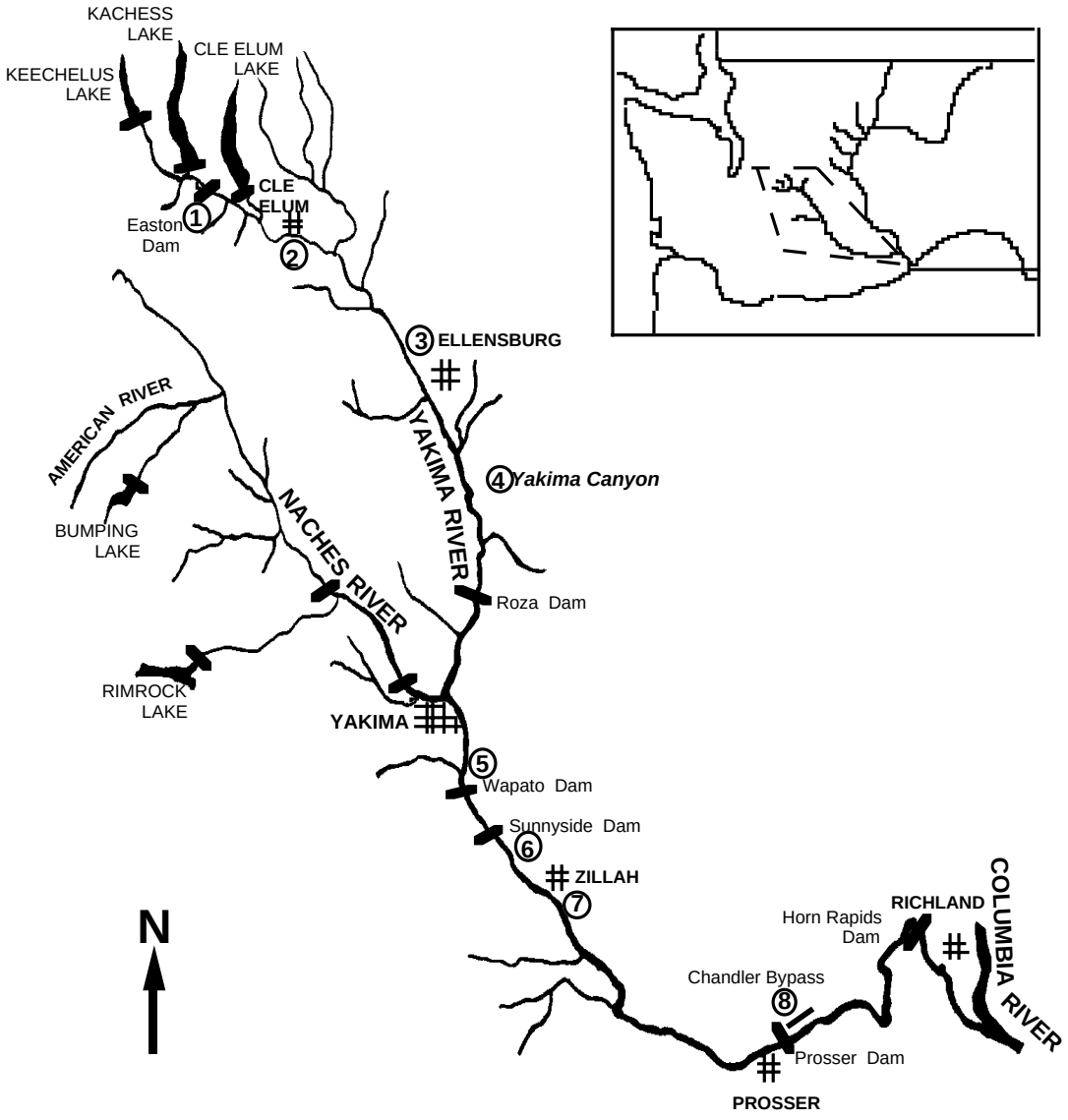

Figure 1. Map of the Yakima River basin. Sites where chinook salmon juveniles were sampled are indicated with a circled number.

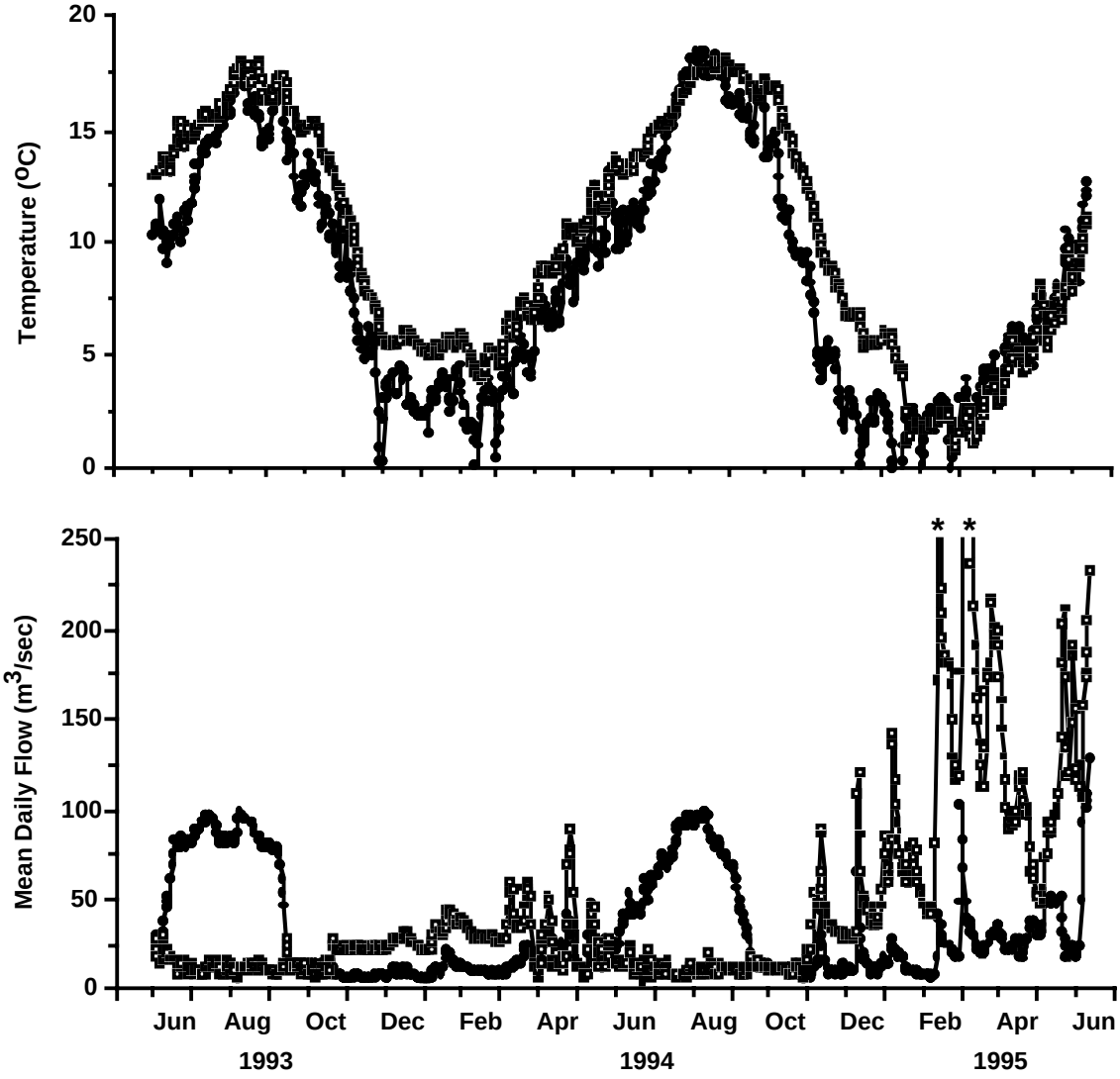

Figure 2. Daily water temperatures measured in the Yakima River from June 1993 through May 1995. Filled circles show temperatures from Cle Elum (upper river) and open circles show temperatures measured at Parker (lower river). The lower panel shows mean daily flow in the Yakima River from June 1993 to May 1995, measured at Cle Elum in the upper river (filled circles) and Parker in the lower river (open cquares). Asterisks denote flooding events in which flow exceed 400 m^3/s.

Table 1. Sampling sites and dates for collection of Spring Chinook salmon (Oncorhynchus kisutch) (brood years 1993-94) in the Yakima River, WA. See Fig. 1 for location of sampling sites. 1=Easton Dam, 2=Cle Elum, 3=Ellensburg, 4=Yakima Canyon, 5=Union Gap, 6=Sunnyside, 7=Zillah, 8=Zillah migrant fish, 9=Chandler migrant fish. Fish were collected by either electroshock(*), beach seine (**), fyke net at sample site 8, removal from Chandler bypass holding tank(***), or from separator screens at the Chandler bypass (site 9). Underscore indicates fish were measured for length and weight only.

		Sampling Site Number								
	Date	1	2	3	4	5	6	7	8	9
Brood	6/7/93	27*	40*		16*					
Year	7/26/93		15*		13*					
1993	8/18/93		14(16)*		12#					
	9/15/93		15*		11*					
	10/5/93		15*		12*					
	10/12/93								-0-	
	10/25/93		15*		12*				-0-	
	11/8/93		15*						15(49)	
	11/16/93								3	
	11/30/93							12*		
	12/16/93		15*						8	
	1/10/94		15*					7*	4	
	1/31/94		15*					12*		
	2/22/94		15*					15*		
	3/8/94		15*					15*	2	
	3/22/94		14*					7*		
	4/4/94		7*							
	4/6/94									13***
	4/13/94									15
	4/18/94									11
Brood	6/22/94		15*		6*					
Year	7/12/94	15*	15*							
1994	8/4/94		15*							
	8/25/94		15*		8*					
	9/8/94			8*		9**				
	9/14/94	10*	15*		15(8)**					
	10/6/94		13*		9#					
	10/19/94					15(16)**			-0-	
	10/27/94		15*						-0-	
	11/2/94						15*			
	11/15/94	15*	15*	15*		15(26)**			3	
	12/20/94		13*				15*			
	1/25/95		10*							
	2/8/95		10*	8*			11*			
	3/1/95	6*	6*							
	3/16/95		7*			12*				
	3/29/95		12*	6*		10*		13**		
	4/6/95					11**		15(27)**		
	4/20/95					15**		15**		15
	4/26/95									15
	5/1/95							15**		15

collected from the bypass facilities at Chandler Dam (April - May) in the town of Prosser (rk 75) (Figure 2).

Capture Methodology

Electrofishing (Smith Root Model 12-A POW backpack Electrofisher, Smith Root Inc., Vancouver, WA) was used to collect fish both above and below Yakima, primarily from around boulders in rip-rapped banks or among woody debris. Beach seining (30m x 2m seine with 1 cm mesh wings and a 0.3 cm mesh bag) was conducted primarily below Yakima, at sites characterized by slack-water eddies below large gravel bars. On certain dates, fish in the river below Yakima were collected by electro-fishing rather than beach seine, this was necessitated by high water conditions (Table 1). During the fall of 1994 we captured fall migrating fish in a fyke net set in a side channel near the town of Zillah. Fish species commonly captured along with chinook salmon above Yakima included juvenile rainbow trout (*O. mykiss*) and sculpin spp (*Cottus spp.*). Fish commonly collected below Yakima included redside shiner *(Richardsonius balteatus*), dace (*Rhinichthys* spp), chiselmouth (*Acrocheilus alutaceus*), northern pikeminnow (*Ptychocheilus oregonensis*) and peamouth (*Mylocheilus caurinus*).

Physiological Sampling

On each sampling day, within a given stretch of river, 6 to 15 fish (depending on collection success) were collected within approximately two hours. All tissue samples were collected on site within 90 minutes of capture of the last fish. Fish were individually anesthetized (buffered 0.05% tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, WA)), measured for fork length (mm) and weight (g), and visually assessed for smolt development (1 = parr, 2 = transitional, 3 = smolt) (modified from Gorbman *et al*., 1982). Blood samples were collected from severed caudal vessels (heparinized Natelson tubes (VWR Scientific)), centrifuged, and stored on dry ice (as were all tissue samples). Gill tissue was collected as described by Zaugg (1982). The liver was removed, weighed (for calculation of HSI), and flash frozen. The sex and stage of sexual development was noted and stomach fullness was graded on a scale from 0 (empty) to 7 (distended). Carci were individually bagged and stored. All samples were stored at -80^0 C until analyzed.

Laboratory Analysis

Plasma T_4 values were determined according to the method of Dickhoff *et al.* (1982). Plasma insulin like growth factor-I (IGF-I) was quantified as described by Moriyama *et al.* (1994). Gill Na^+ K^+ ATPase (ATPase) activities were measured as described by Schrock et al. (1994). Liver glycogen was measured as described by Plisetskaya *et al.* (1994). Whole body lipid levels were determined by the method of AOAC (1975) using methylene chloride for extraction.

Data Analysis

Polynomial regression was performed on the mean value of characters for each sampling date and location according to Ryan (1997) using Statview II (Abacus Concepts, Cupertino CA). All data were $\log_e$ transformed prior to analysis, significance levels and correlation coefficients are reported in figure legends. The polynomial equation calculated for each character was determined by an iterative process, starting with lower order equations and advancing to higher order equations until a non-significant result was obtained (t-test). Condition factor was calculated as: condition factor = [weight (g) / length $(mm)^3$] X 100,000.

RESULTS

Temperature and flow

The Yakima River underwent distinct seasonal changes in temperature and flow, with variation between years (Figure 2). Temperature reached 17 to 18ºC in July and August and steadily declined through the fall, reaching seasonal lows of 0ºC December through February. Temperatures increased in each year, beginning in March and continuing throughout July. Flow at Cle Elum was marked by high seasonal discharge, June through September, primarily due to water released from upstream reservoirs (Figure 2). Flow decreased markedly in September. Flow at Parker was stable through much of 1993 and 1994. The winter and spring of 1995 were marked by relatively high flow and several large flooding events.

Size and morphology

Length and weight were both modeled by 3rd order polynomials (r^2>.70, Figure 3 and 4). Size increased from June to October, changed little from October through February, and then increased again from March through May. Migrants captured at Chandler in April - May (> 120 mm and > 20 g) were considerably larger than other fish captured by electro-fishing or seine in the spring (100 - 120 mm and 10 - 17.5 g). October - February sizes were similar between years ranging from 90 - 110 mm and 7.5 - 15g respectively.

Condition factor showed a complex pattern of change which was best modeled with a 4th-order equation (r^2=0.45). Condition factors were high in August - September (1.10 - 1.25) and decreased to low values in January (1.00 - 1.10) (Figure 5). Condition factors increased in some samples taken in March - April (>1.15). The condition factors of Chandler migrants were uniformly low (<1.05).

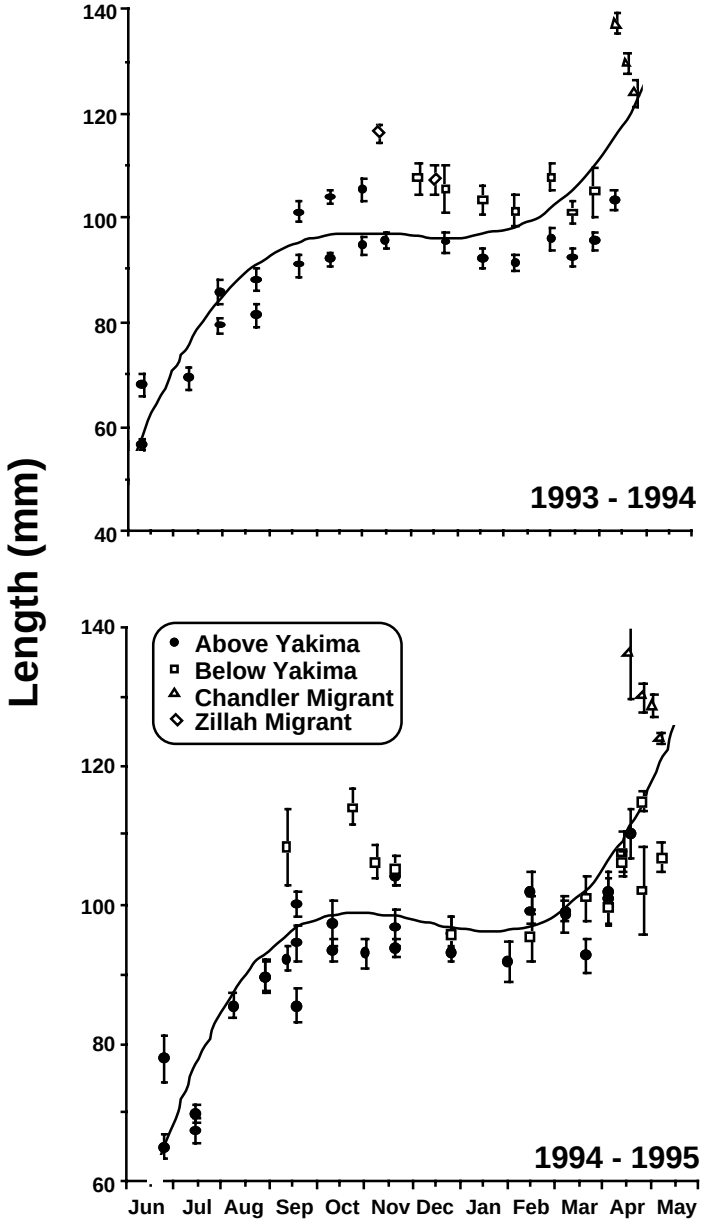

Figure 3. Length of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.0001$, $r^2=0.79$, 1993 - 1994; $p=.0001$, $r^2=0.73$, 1994 - 1995).

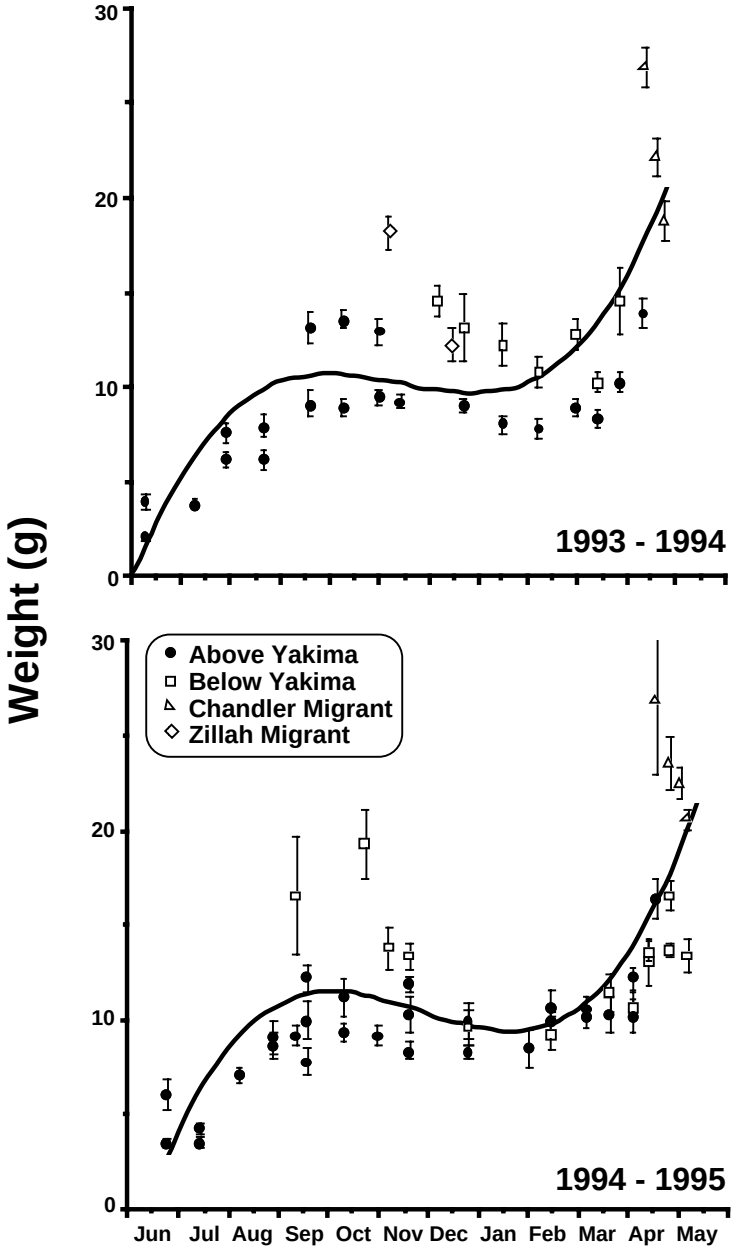

Figure 4. Weight of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass (p=.0001, r^2=0.76, 1993 - 1994; p=.0001, r^2=0.74, 1994 - 1995).

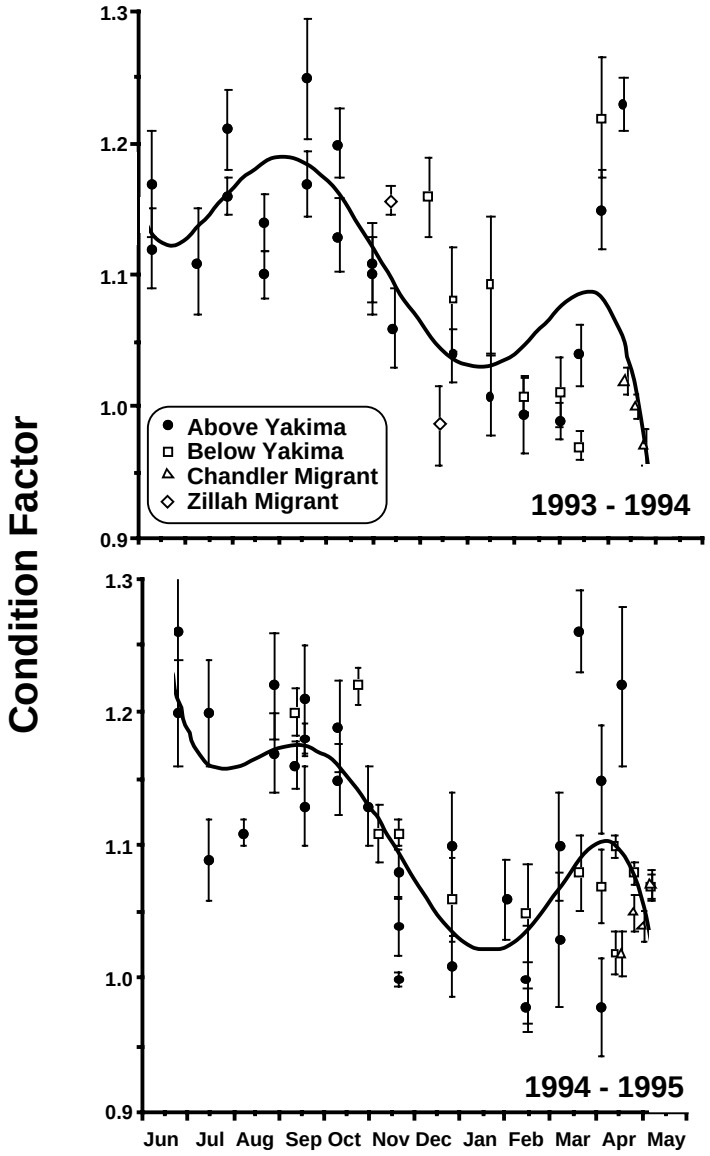

Figure 5. Condition factor of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.009$, $r^2=0.36$, 1993 - 1994; $p=.0001$, $r^2=0.43$, 1994 - 1995).

Energy reserves

Whole body lipid showed a seasonal profile similar to that of condition factor (Figure 6) and was best modeled with either a 3rd or 4th order equation (r^2=0.53 1993 - 1994, r^2 =0.64 1994 - 1995). Values were highest in late summer (August - September) (5 - 8%) and decreased through the winter (January - February), reaching lows of 2 - 3.5%. In both winters the polynomial regression indicated an inflection in January, with average body lipid levels increasing to almost 4% in February - March at several sites during both years. Average body lipid levels of Chandler migrants were always less than 3%.

The stomach fullness data did not fit any regression equation well (Figure 7, r^2=0.30 1993 - 1994, n.s. 1994 - 1995). There was an approximate seasonal pattern, with highest stomach fullness indices found in late summer (August - September) and spring (March - April). For winter of 1994 - 1995 (15 November through 15 February) 71 of 136 individuals sampled (52%) had stomach fullness scores of 2 or less, while only 18 had scores of 5 or higher (13%). In contrast, for spring (16 February through 15 April) 30 of 154 individuals (20%) had scores of 2 or less and 48 individuals had scores of 5 or better (31%). Generally, there was a great deal of variation in stomach fullness in the spring, while stomachs were mostly empty or near empty in the winter.

Liver glycogen values were quite variable and there was no consistent seasonal pattern. It is noteworthy that all samples taken from smolts in the lower river and at Chandler in April - May of 1995 had quite low values (< 5 mg/g)(Figure 8). No significant regression model could be found for the 1993 - 1994 data and a binomial model was produced for 1994 - 1995, but the fit was poor (r^2=0.3).

Hepato-somatic indices showed a complex pattern of change in each year which was best modeled with a 4th order polynomial (Figure 9, r^2=0.40). High values were found in the spring (March - April), while lowest values were found in the winter (December - January) for the 1993 - 1994 data or in late fall - winter (August - October) for the 1994 - 1995 data.

Appearance and smolt physiology

Body appearance changed dramatically in the spring and was best modeled with a 3rd or 4th order equation (Figure 10, r^2=0.50 1993 - 1994, r^2=0.84 1994 - 1995). All migrants taken at Chandler were scored as 3's, meaning they were strongly silvered, had no parr marks, peripheral fins were colorless, and had strong black margins on the caudal fin. This was in contrast to samples taken in mid-winter when parr marks were distinctly visible and peripheral fins were bright yellow. In each year body appearance also changed in the fall; some fish collected at that time had silvery bodies and parr marks were obscured; however, peripheral fins never cleared and no black fin margins were observed.

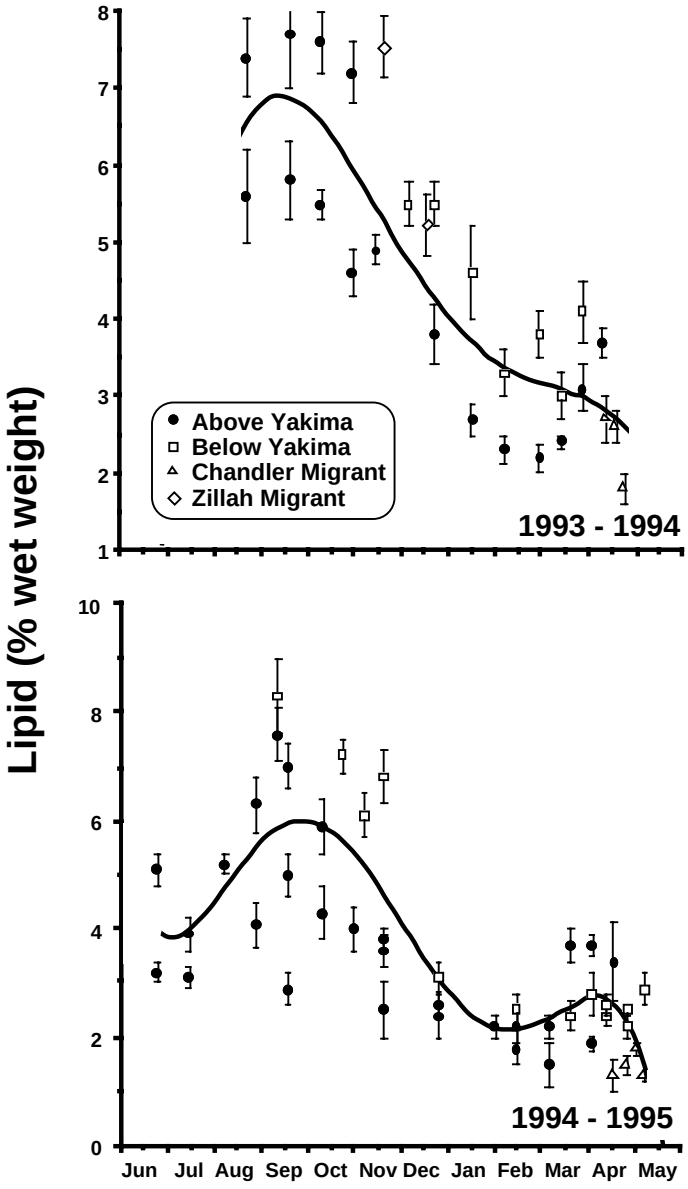

Figure 6. Body lipid composition (% wet weight) of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.001$, $r^2=0.53$, 1993 - 1994; $p=.0001$, $r^2=0.64$, 1994 - 1995).

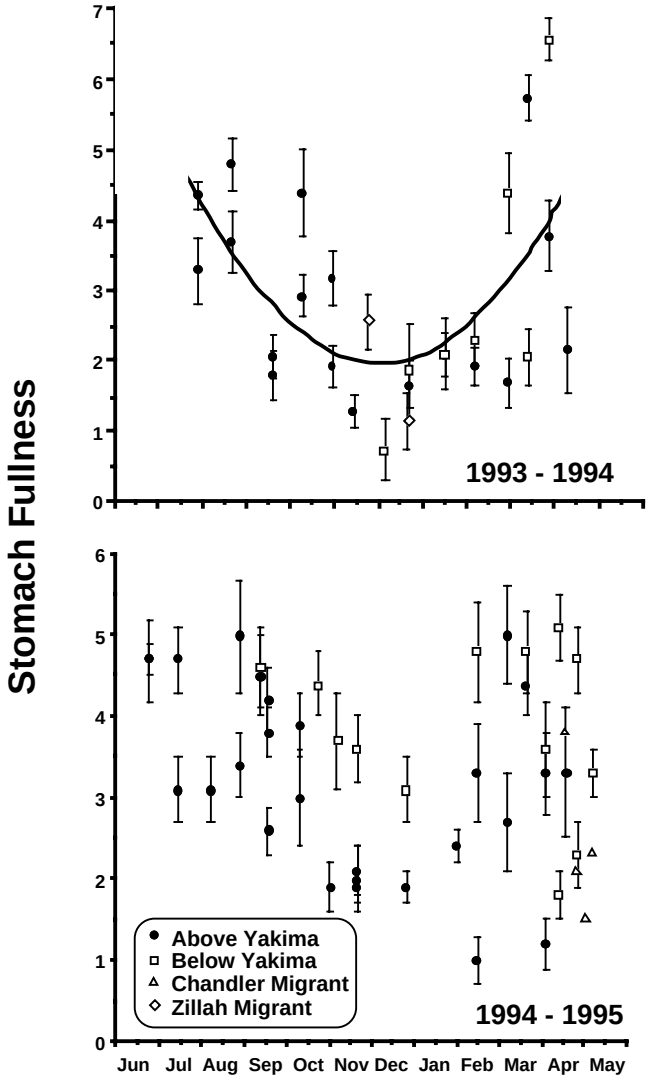

Figure 7. Stomach fullness of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.008$, $r^2=0.30$, 1993 - 1994; n.s. 1994 - 1995).

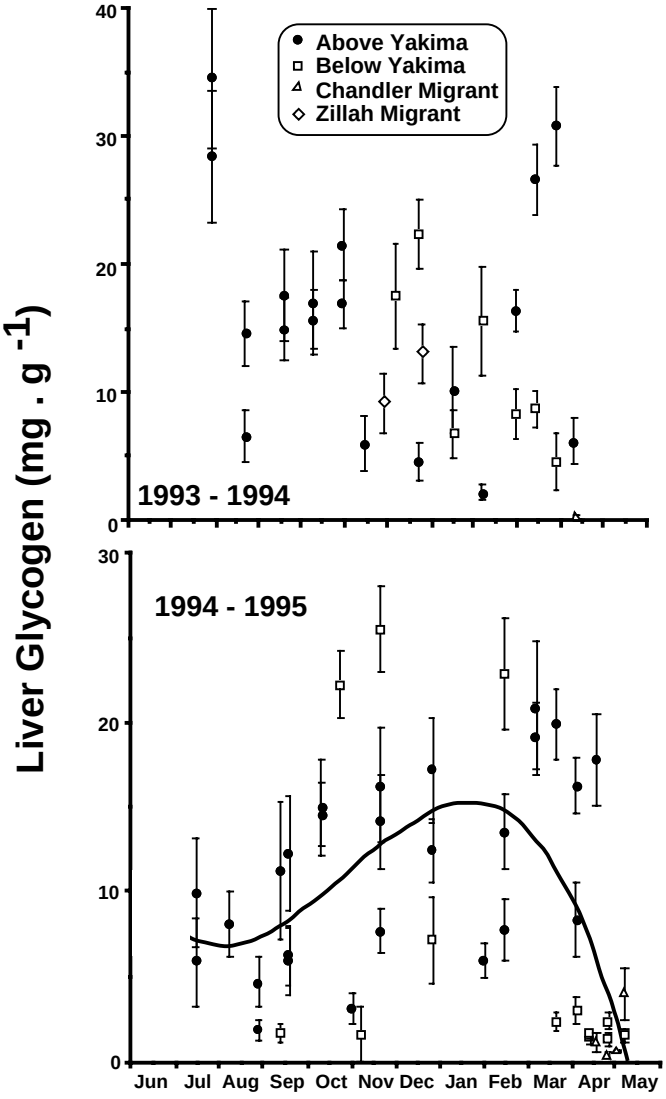

Figure 8. Liver glycogen content of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass (n.s. 1993 - 1994; p=.0001, r^2=0.30, 1994 - 1995).

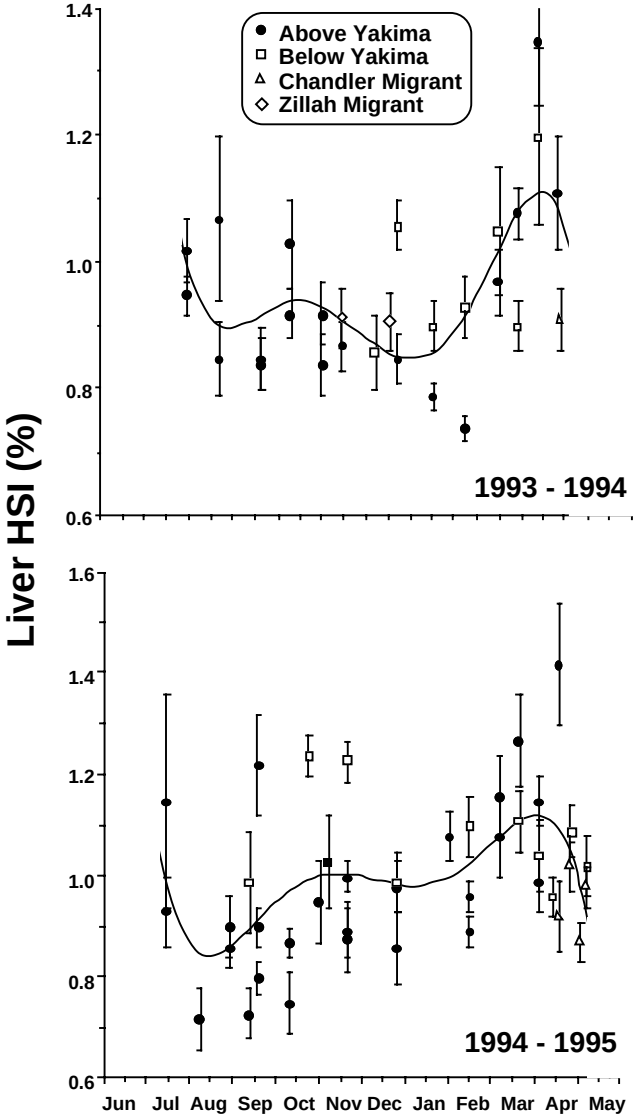

Figure 9. Hepato-somatic index of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.007$, $r^2=0.40$, 1993 - 1994; $p=.03$, $r^2=0.40$, 1994 - 1995).

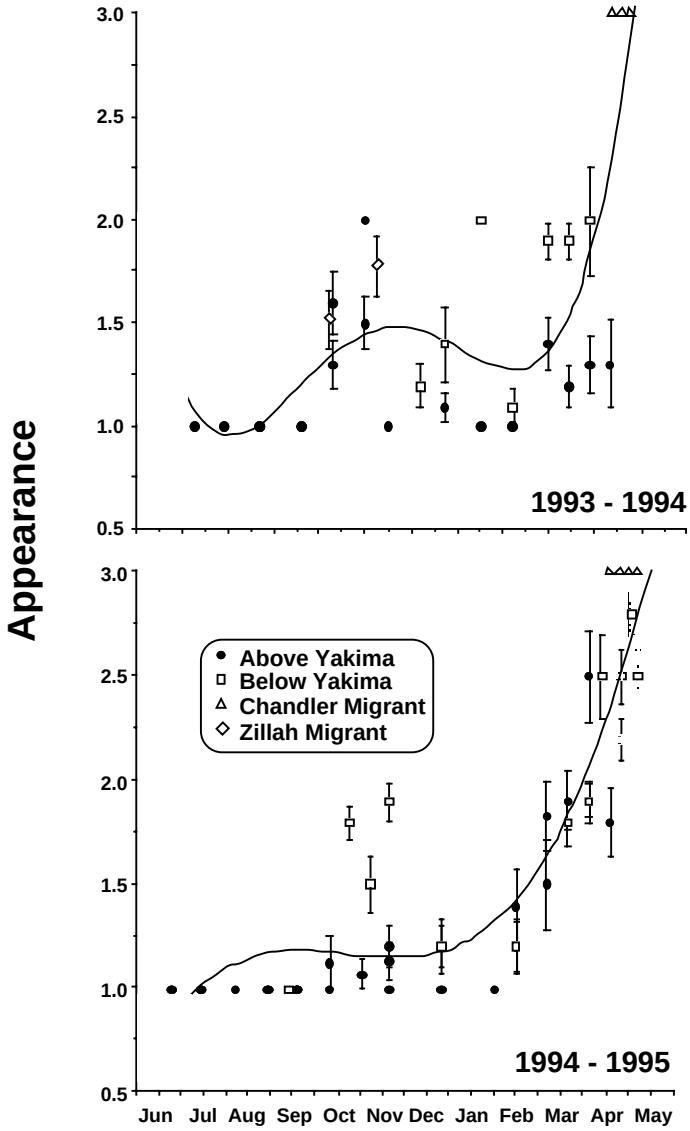

Figure 10. Body silvering of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.0004$, $r^2=0.50$, 1993 - 1994; $p=.0001$, $r^2=0.84$, 1994 - 1995).

Seasonal plasma thyroid hormone profiles were different between years (Figure 11). A four factor polynomial was fit to the 1993 - 1994 data, with a poor fit to the equation (r^2 = .34); while, a three factor polynomial was fit to the 1994 - 1995 data, with a better fit (r^2 = .54). The main difference between the years lies in the high T_4 values found in fish in the spring of 1995 in lower river and at the Chandler by-pass as compared to those sampled in 1994. Furthermore, an additional inflection point was found in the 1993 - 1994 data in March, which appears to be strongly driven by one of the three data points generated by sampling fish at the Chandler by-pass. Finally, for 1994 - 1995, fish sampled below Yakima in the spring had much higher T_4 values than those above Yakima.

Plasma IGF-I levels increased more than two-fold from February through May and mean values were best modeled with either a 3rd or 4th order equation (Figure 12, r^2=0.45 1993 - 1994, r^2=0.54 1994 - 1995). The highest values were associated with samples taken from migrants at the Chandler bypass. The model for 1994 - 1995 displayed an additional inflection point in July - August, which was strongly influenced by one data point in June.

The gill ATPase data were fit with either a 2nd or 3rd order polynomial and displayed a poor to moderate fit (Figure 13, r^2=0.34 1993 - 1994, r^2=0.57 1994 - 1995). Gill ATPase increased strongly in the spring of each year, with values increasing from < 4 μmole PO_4/mg pro/hr, to > 10 for fish sampled at the Chandler bypass. Values of samples taken from Chandler migrants were greatly elevated over other samples in 1993 - 1994. In 1994 - 1995 values from Chandler migrants were similar to that of samples taken from fish seined in the lower river. There appeared to be some fish displaying elevated ATPase values in the summer - fall of 1994 - 1995; however, these levels did not approach those found in the spring.

DISCUSSION

We conducted a simple descriptive study, examining endocrine and physiological characters of juvenile spring chinook salmon in the Yakima River. However, analysis of the data was not simple. These fish displayed distinct seasonal physiological changes, yet; the response of different physiological characters varied in direction (positive or negative), magnitude, and temporal pattern. Overall, we are most interested in comparing and discriminating similar patterns of change between different physiological characters and examining the relevance of these patterns within an organismal context. We tried a number of different methods of grouping and discriminating between fish collected at different places and on different dates. Based on these analysis, we chose to model the data as representative of one population reacting to rather large seasonal variations in photoperiod and temperature. The fit of the data to the polynomial regression model may be regarded as an assessment of how well our assumption was met. Some characters, such as stomach fullness and liver glycogen, did not show a significant relation to season, these parameters were probably responding to smaller scale variation which we couldn't identify. Undoubtedly there are environmental and genetic differences between fish rearing in different parts of the river; however, our sampling design simply gives us no power to discriminate these differences so we will spend little effort in discussing them.

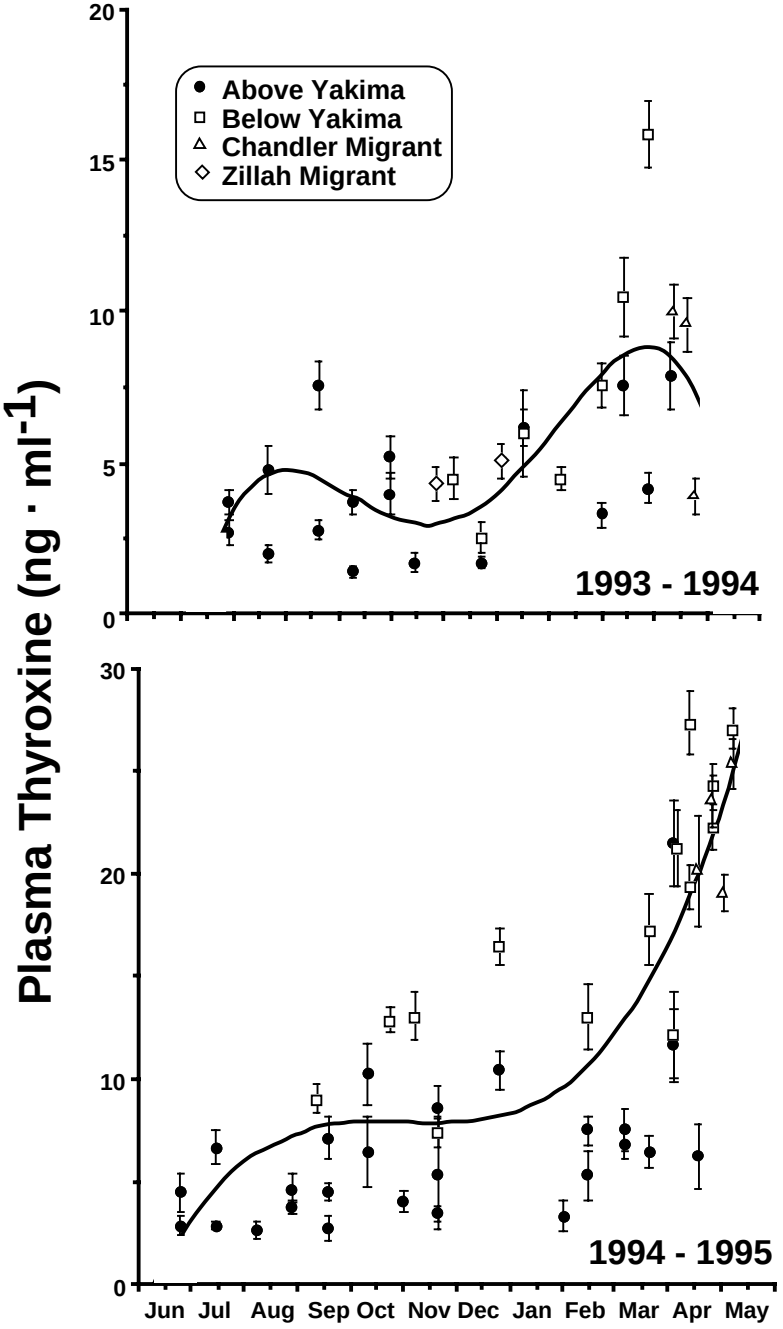

Figure 11. Plasma thyroxine concentration of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.01$, $r^2=0.34$, 1993 - 1994; $p=.0001$, $r^2=0.54$, 1994 - 1995).

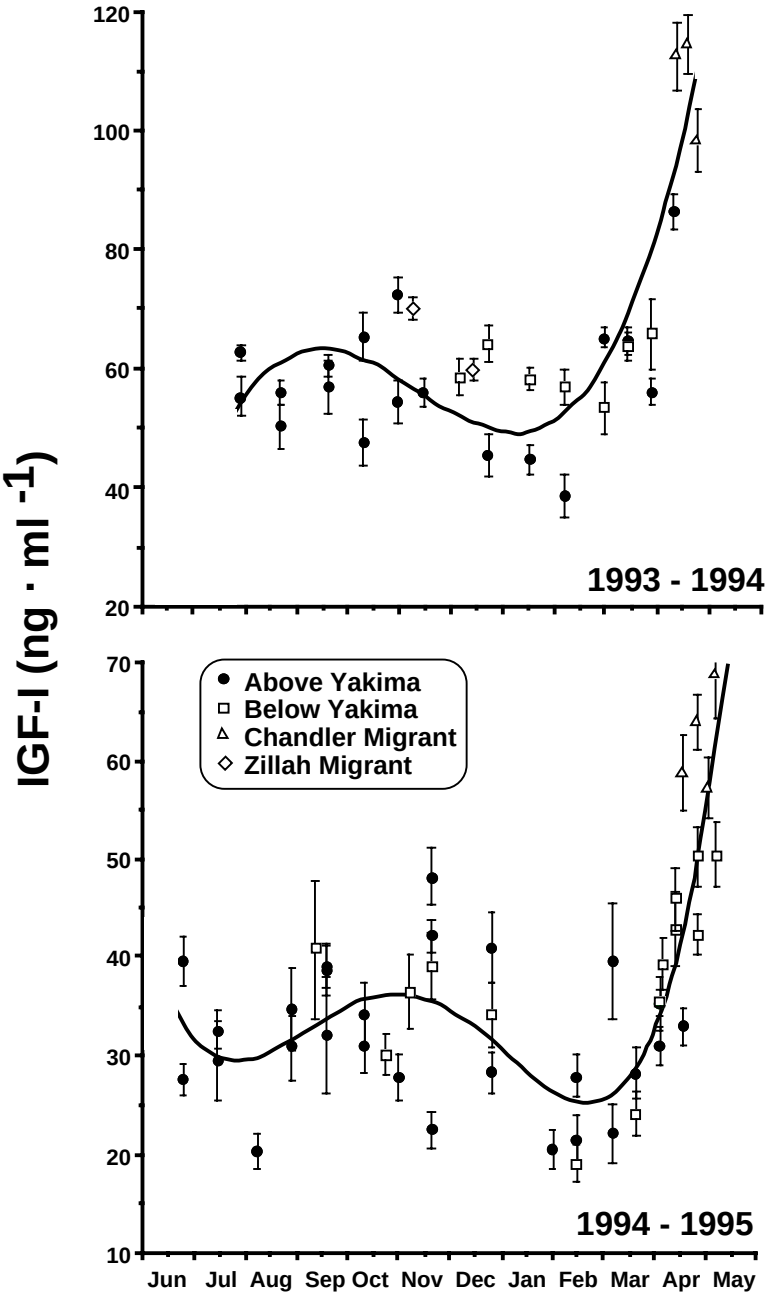

Figure 12. Plasma insulin-like growth factor-I concentration of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass (p=.001, r^2=0.45, 1993 - 1994; p=.0001, r^2=0.54, 1994 - 1995).

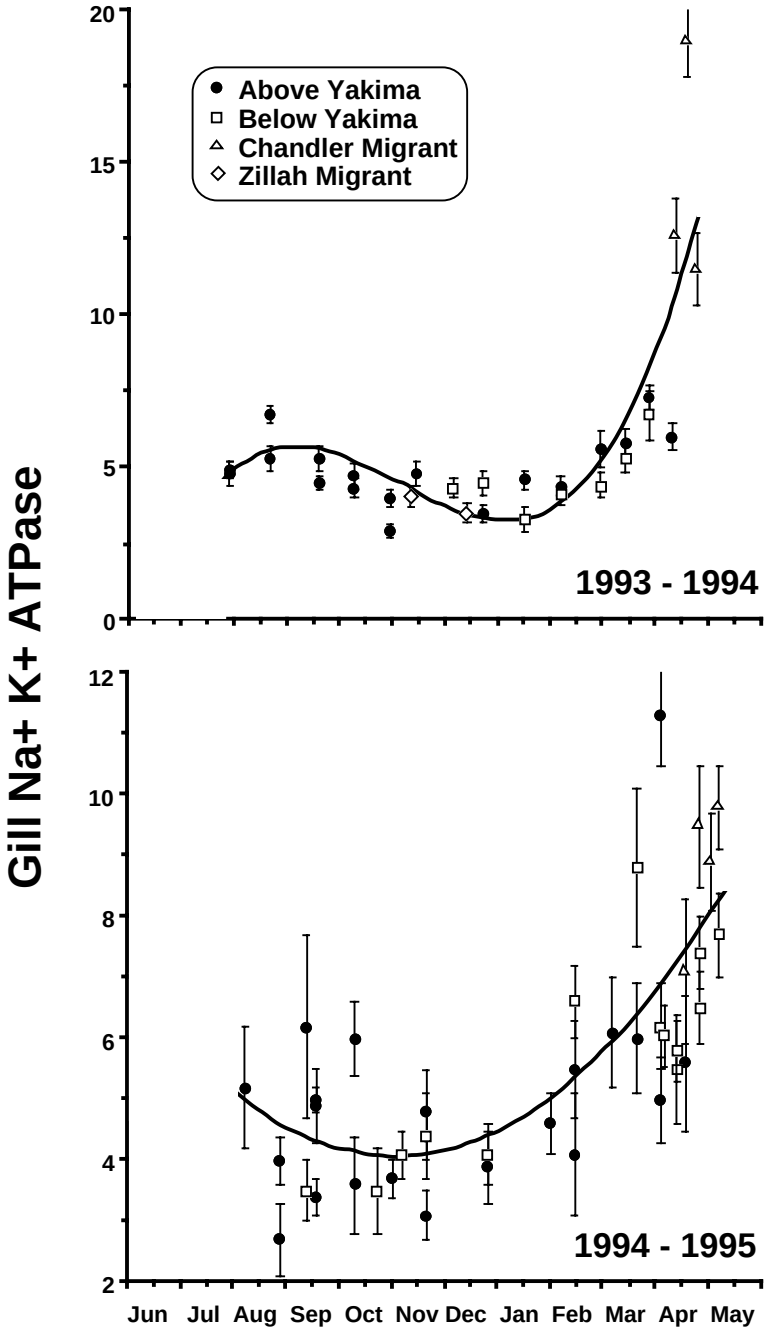

Figure 13. Gill ATPase activity of juvenile chinook salmon captured in the Yakima River June 1993 - May 1994 (upper panel) and June 1994 - May 1995 (lower panel). Filled circles represent values for fish collected above the city of Yakima, open squares represent values for fish collected below the city of Yakima, and open triangles represent values for fish collected at the Chandler by-pass ($p=.004$, $r^2=0.34$, 1993 - 1994; $p=.0001$, $r^2=0.57$, 1994 - 1995).

Energy reserves, growth and the winter-spring transition

There was little or no growth from October through February, as might be expected based on temperature profile and previous work on salmonids (Figure 2, Elliott 1994, Weatherly and Gill 1995). Similarly, Levings and Lauzier (1991) found little change in size of yearling chinook salmon during winter in the Fraser River. Studies of other free-living juvenile salmonids have found seasonal growth curves that closely follow seasonal temperature changes (Jensen 1990, Elliott 1994, Lobon-Cervia and Rincon 1998). Mean size of outmigrant smolts found in this study are similar to those reported by Major and Mighell (1969) and Fast et al. (1991) in the Yakima River. Although it is difficult to compare among studies of different years, with different sampling methods, outmigrating Yakima River smolts appear to be larger than smolts from other interior Columbia River chinook salmon populations, where smolts are generally less than 120 mm (Bjornn 1978, Lindsay et al. 1986, 1989, Burck 1993).

The effects of winter conditions, or differences in winter conditions, on metabolism, energy reserves, and mortality of juvenile chinook salmon are relatively unexplored. Several studies of juvenile salmonids have reported decreases in condition factor and/or lipid through the winter (Cunjak and Power 1986, 1987, Berg and Bremset 1998). Rogers et al. (1989) reported extractable lipid levels (dichloromethane:hexane) of 1.7 - 6.0 % from chinook salmon juveniles captured in the Fraser River in December, values comparable to those found in the Yakima River. Studies of juvenile rainbow and brook trout *Salvelinus fontinalis* and Atlantic salmon suggest that winter mortality may be greater than 50% (Meyer and Griffith 1997, Cunjak et al. 1998). Our data suggests that free-living juveniles lose energetic reserves during winter, and implies that severe winters or scarce food supplies during the previous summer-fall could impact overwinter survival.

We found low stomach fullness scores in the winter, including a rather significant proportion of empty stomachs. Stomach clearance times slow down at low temperature, so it is not unreasonable to suggest that these fish are not feeding much in the winter. This supports our supposition that Yakima River chinook salmon juveniles did not grow much and experienced an energetic deficit during the winter. During other seasons stomach fullness was highly variable, which may be due to daily sampling time, since several studies have noted feeding activity of juvenile salmonids is variable through the day (Johnson and Johnson 1981, Sagar and Glova 1988). The logistics of field collections at several sites eliminated the possibility of sampling at a set time of day for fish at all locations. In addition, there were almost certainly differences in the quantity and quality of food available at different sites due to longitudinal differences in physical environment, prey availability and potential competitors in the Yakima River (Fend and Carter, 1995, Leland 1995). Overall, there appears to be broad seasonal changes in the feeding of juvenile chinook salmon in the Yakima River, but our data is neither accurate nor precise enough to support further conclusions.

It is apparent from the shape of the regression models that there is little concordance between most of characters associated with feeding, metabolism and energy storage (gut fullness, liver glycogen, HSI, body lipid and condition factor). Given differences in daily sampling time and stomach fullness, based on other studies, it is not unexpected that we should find large variation in liver glycogen and HSI values (Boujard

and Leatherland 1992). Liver glycogen values found in this study were variable and no seasonal trend was apparent. However, the very low values found in migrating smolts at Chandler are notable. A seasonal pattern in HSI was apparent, with peaks in the fall and spring. Hepato-somatic indices are valuable indicators of energetic status in Atlantic cod *Gadus morhua* (Couture et al. 1998), which in contrast to salmonids, store much of their lipid reserves in their liver (Zhou et al. 1996). Thus, HSI may not provide a good signal of energetic status for juvenile salmon.

One of the most striking, yet unexpected, findings from this study was an increase in anabolic characters in the spring (February-April). The catabolic response of juvenile salmon in the winter was expected (the result of low temperatures and little food); in turn, an anabolic increase was expected in the late spring, following increased temperatures and food supply. Surprisingly, body lipid, condition factor, and size increased in February - March, when water temperatures were still low (5^{o}C) and we anticipated food supplies would be limited. However, stomach fullness appeared to increase in February - March, suggesting that increased condition factor and body lipid values were reflective of changes in feeding activity and energetic status. In addition, increases in body size suggest that growth had occurred. Finally, it is unlikely that the increase found in plasma IGF-I (early April) could occur in fish that were not nutritionally replete (Perez-Sanchez et al. 1995, Duan et al. 1995, Beckman et al. 1998).

There are few other data on the anabolic status of free-living juvenile salmonids during the spring. Cunjak and Power (1986) found an increase in lipid between March and May in juvenile Ontario brook trout. Berg and Bremset (1998) found an increase in body lipid from April to June in juvenile Atlantic salmon and brown trout *Salmo trutta* in Britain. In addition, Tveiten et al. (1996) found increases in appetite and growth rate of yearling arctic charr *S. alpinus* in April and May, even though the fish were held at a constant 4^{o}C. Koskela et al. (1997) found that brown trout will feed at 2^{o}C, and growth was significantly increased at 4 and 6^{o}C, temperatures similar to the Yakima River in spring. Finally, Forsberg (1995) reported on seasonal growth of Atlantic salmon held in land-based culture facilities in Norway. He found significant increases in growth during February, March or April at different facilities and suggests that "growth was more closely linked to seasonal changes in photoperiod than to water temperature". Together, these studies suggest that salmonids may initiate anabolic processes at low temperatures in the spring in response to changing photoperiod.

Growth hormone has been shown to be photoperiod responsive (Bjornsson et al. 1989, 1995, McCormick et al. 1995) and increases in GH have been found as early as March in fish held under natural photoperiods. In addition, an increase in plasma insulin has been found in coho salmon *O. kisutch* as early as February (Plisetskaya et al. 1988) and in Atlantic salmon smolts in March (Mayer at al. 1994). Increases in either GH or insulin have been suggested to be related to promote growth (Mommsen 1998). Thus hormones capable of inducing growth increase in the spring in captively reared fish, and may also occur in free-living fish.

We do not know whether an increase in energy resources in the spring is a situation unique to the Yakima River in the two years in which we sampled or whether it is a common feature in free-living chinook salmon juveniles. There are several other possibilities we have to consider to explain our findings. There may have been changes in fish behavior or habitat preference, associated with the change in season, that resulted

in fish with different physiological status becoming more available for sampling in February - April. We have no data with which to dispute this supposition. However, it seems unlikely that there was an area in the river in which fish did not experience "winter-like" conditions, thus we would expect decreased lipid and condition factor in all fish. In addition, increases in body lipid and condition factor appear prior to the spring increase in size. If fish in "better condition" were becoming more accessible to our capture methods in March we might expect that they would be larger.

Perhaps combining data from fish rearing in upper and lower-river areas drives the dynamic found in our data. In most cases values were distributed both above and below the regression line in February - March, suggesting that samples from both locations contribute to increases in condition factor and lipid in March. It is apparent that some fish, that are not necessarily geographically segregated, had higher lipid levels and condition factors than fish found in mid-winter. Finally, the lipid depletion rate found from October through February could not be sustained through April as body lipid composition would approach zero, a physiological impossibility. Similarly, the decrease in condition factor levels off in February and March, regardless of a few high values that may cause the regression model to show an actual increase. We thus discount the possibility that sampling methods or local geographic differences in fish sampled from the river generated the inflection in the polynomial regression found in February and March. The data support an increase in condition factor and lipid, suggesting that juvenile salmon in the Yakima River shift from a catabolic to an anabolic state in the early spring.

Spring smolting

This is the first report of plasma IGF-I values from free-living juvenile salmonids. We found a large increase in IGF-I in the spring associated with smolting and downstream migration. Values increased 200 - 300% from resident parr in February to migrating smolts in April - May. These changes are much larger than have been previously documented in relation to smoltification in captively reared chinook salmon, either in hatchery or laboratory situations (Beckman and Dickhoff 1998, Beckman et al. 1998, Beckman et al. in press, Silverstein et al. 1998). Komourdjian et al. (1976) and Dickhoff et al. (1997) have both discussed the possibility that GH or the GH/IGF-I endocrine axis plays a key role in stimulating and coordinating the process of smolting. McCormick and Bjornsson (1994) reported high plasma GH levels in free-living, migrant Atlantic salmon smolts and several authors have described seasonal increases in GH associated with smoltification (Bjornsson et al. 1989, 1995, McCormick et al. 1995). The IGF-I levels measured in this study, coupled with the GH levels measured by McCormick and Bjornsson (1994), suggest that the GH/IGF-I endocrine axis is highly stimulated in migrating smolts, and reinforce the notion that this axis and growth are important elements of smolting (Dickhoff et al. 1997, Beckman et al. 1998).

The spring changes in plasma T_4 , IGF-I and gill ATPase were relatively small in fish taken above Yakima compared to migrating smolts at Chandler. Conversely, condition factor, liver glycogen and body lipid values were much lower in fish sampled at Chandler than in fish sampled above Yakima. We attribute these changes to the smolting process; however, we did not attempt to discriminate differences between parr and

smolts. Numerous studies have demonstrated that parr and smolts differ in almost any physiological character one wants to examine. Instead, our data, grouping parr and smolt, demonstrates that smolting in naturally rearing fish is a temporal process, requiring weeks to accomplish (at least in a populational view). In addition, this study, along with several others (Zaugg et al. 1985, Muir et al. 1994, Schrock et al. 1994, Haner et al. 1995), shows smolting is a spatial process, physiological changes intensifying as fish move downriver. We have thus attempted to define the temporal and spatial trajectory of physiological changes accompanying smolting. Our analysis suggests that endocrine and physiological changes occur in concert with migration in naturally-reared salmon, rather than preceding migration. This conjunction of behavioral and physiological change found in naturally rearing and migrating fish may make comparisons to fish held in hatchery raceways or laboratory tanks tenuous.

Energy reserves and smolt migration

Our observations of migrating smolts ended at the Chandler bypass, 75 km from the confluence of the Yakima River with the Columbia River and a further 540 km from the Pacific Ocean. Smolts captured at the Chandler bypass displayed depleted liver glycogen and body lipid stores, after they had traveled no further than 250 km (Easton to Chandler), at most 30% of their journey. It is difficult to understand how such a short migration could result in severe depletion of energy stores, and how the fish could support continued metabolic demand through the remainder of their journey. Vijayan et al. (1993) found only moderate decreases in liver glycogen for juvenile coho salmon continuously swam and fasted for three weeks. The nearly unmeasureable liver glycogen levels found in migrant smolts at the Chandler bypass suggest that exercise alone may not explain these reduced glycogen levels.

A number of reports have documented increased metabolic rates in smolting salmon (for review see Hoar 1988). This increased metabolic rate might be directly associated with an increase in circulating hormones with catabolic properties. Several experiments have shown that GH treatments in salmonids results in reduced lipid levels or condition factor (Danzmann et al. 1990, O'Conner et al. 1993). In addition, thyroid hormone and cortisol are lipolytic in salmonids (Sheridan 1986). The combined effect of high plasma GH, cortisol, and thyroid hormone is inevitably lipid depletion - regardless of the activity level of the fish. The high plasma levels of lipolytic hormones measured in migrating smolts (McCormick and Bjornsson 1994), coupled with the quite low lipid and glycogen values found in migrating Yakima River smolts, suggest that the lipolytic status of smolts has been underestimated in many laboratory experiments. These observations suggest we have not yet determined the causal mechanisms (endocrine or exercise) leading to the high metabolic rate and catabolic status of migrating smolts, leaving it difficult to assess the physiological impacts of environmental alterations on these animals.

Fall migration

An autumnal migration from headwater areas to lower river reaches or mainstem areas appears to be characteristic of many interior populations of Columbia and Fraser

River chinook salmon (Bjornn 1971, 1978, Lindsay et al. 1986, 1989, Murray and Roseanau 1989, Levings and Lauzier 1991, Burck 1993). In addition, several studies demonstrated that chinook juveniles may smolt in the fall (Ewing et al. 1980, Beckman and Dickhoff 1998). Our data suggest that appearance, T4, IGF-I and gill Na^+ -K^+ ATPase all show some signs of increased values in September - October, either by an inflection point in the regression model or by increased variation in data around the regression line. However, these data are difficult to evaluate; it is not clear whether part of the population is smolting, or whether there is some seasonal physiological change, which may be associated with fall redistribution movements.

The majority of Yakima juveniles are not smolting and emigrating to the ocean in the fall. The large outmigration of yearling smolts the following spring clearly argues against this (Fast et al., 1991). In addition, no adult spring chinook from the Yakima River (>1,000 samples) were found to possess ocean-type scale characteristics (found in fish which migrate to the ocean as sub-yearlings) (Knudsen 1991). These data provide little or no evidence for fall smolting in naturally rearing Yakima chinook salmon, thus we can not ascribe fall changes in physiology to smolting.

Fall re-distributions have been described as a search for appropriate over-wintering habitat (Bjornn 1971, Hillman et al. 1987), though this behavior may not be universal within a population. In the Yakima River, some fish rear year-round at or above Cle Elum, while others move several hundred kilometers downriver in the fall (Fast et al. 1991). Migratory behavior may be genetically fixed, Bradford and Taylor (1997) showed that individual chinook salmon fry display either migratory or non-migratory behavior in the spring following emergence and further, that individuals from different populations possessed different tendencies to migrate. However, fall migration might also be a conditional strategy, dependent on habitat or the fishes physiological status. We did not find dramatic physiological differences between fish sampled above Yakima and below Yakima (which had presumably recently migrated downstream) in the fall. Fish captured in the lower river in the fall did tend to be larger and have greater lipid deposits than fish collected in the upper river, suggesting that fall migrants were not energetically stressed. Thus, while it appears to be a relatively common trait in juvenile spring chinook salmon, a physiological relation to fall in-river migration is not apparent.

Annual Variation

The winter of 1993 - 1994 was relatively warm with relatively low flow. In contrast, the winter of 1994 - 1995 was colder and punctuated with several major floods. We did not conduct a formal test for differences in physiology between years, as our data provided little power to determine year to year variation because of differences in sampling method, date, location, and small number of fish per sample. Holtby et al. (1989) found differences in median date of smolt migration for coho salmon in Carnation Creek (Vancouver Island, BC) of up to 21 days over 17 years of study. This variation was highly related to annual differences in temperature. Similarly, Fast et al. (1991) found differences of up to 18 days in date of 50% passage at Chandler Dam for chinook salmon in the Yakima River (1983 - 1990). Given the large role temperature plays in the physiology of poikilotherms, we might expect physiological differences associated with annual variation in temperature. However, to discern differences would require multi-

year sampling, and large numbers of fish, sampled at a common physiological points (mid-winter, smolt outmigration).

There was some variation in absolute levels of gill ATPase, T4, and IGF-I measured between years. However, the same trend was evident in each year: values were low in the winter and increased in the spring. The slope of the ATPase curve appears shallower in the spring of 1994-95 compared to 1993-94, which is likely due to the greater number of samples obtained in the lower river in the second year. The shape of the T4 curve in 1993-94 appears to be radically different than in 1994-95. The apparent decrease in 1993-94, found in April, is due to the leverage produced by one sample with a low mean value. As stated previously, we attach little physiological significance to inflection points found at the beginning or end or the sampling profile. The important physiological point is that values increased from winter to spring in each year. The IGF-I profiles are essentially identical between years; however, the absolute values measured in 1994-95 are roughly half those found in 1993-94. We cannot at this time ascribe particular significance to differences in absolute values of blood hormone levels. As was shown for thyroid hormones in relation to smolting, the presence of a seasonal change is more important than the absolute level measured (Dickhoff et al. 1982).

Growth and adiposity of naturally rearing vs. hatchery chinook salmon

The seasonal growth patterns of naturally rearing fish may be different than that of cultured fish. A previous study (Beckman et al. in press) of juvenile chinook salmon from Columbia River hatcheries using various water sources showed three different seasonal growth patterns: little growth from November through April (release); continued growth from November through February with an increased growth rate from February to April; and little growth through the winter, then increased growth February through April. In that study spring growth rate was positively related to smolt development and subsequent smolt to adult survival. Given the generally better smolt-to-adult return of wild chinook salmon as compared to hatchery smolts (Lindsay et al. 1989, Fast et al. 1991), the significant early spring growth found in wild fish reinforces the hypothesis that spring growth rate may be important for smolt development and performance. However, it is not yet clear whether a high growth rate promotes smolting. Wild fish go from no growth in the winter, to more rapid growth in the spring. It may be the dynamic change in growth rather than the rate of spring growth that is important to wild fish performance.

Lipid levels in naturally-reared Yakima River fish were strikingly lower than in Yakima River fish reared in an experimental hatchery and fed commercial feed. The cultured fish had lipid levels ranging from 11 - 8% January through March (Beckman et al. 1998), which is commonly found in fish fed commercial feeds (Shearer et al. 1997). The high energy density of artificial feeds and high feeding level in culture promote high adiposity. The significance of the differences in body lipid level between cultured and free-living fish has not been established, thus what constitutes an obese fish in not known. However, the biomedical field has clearly shown the hazards of obesity in humans (Wickelgren 1998) and this suggests that investigation into the effects of high adiposity on the physiology, health and development of captive salmonids may be of interest.

Integrated seasonal pattern

On a seasonal basis, juvenile chinook in the Yakima River appear to go through four distinct physiological states. An anabolic phase in summer - fall, in which fish increase in size and store energy as lipid. A catabolic phase that extends through the winter, in which fish cease growth and deplete energy reserves. A second anabolic phase in February - March, where fish once again begin to grow and replenish energy reserves. Finally, a period (March –April) that exhibits both anabolic and catabolic elements as body size continues to increase; yet, energy reserves are depleted. For continued reference within this paper, we will refer to this as the smolt-associated metabolic state. These phases are illustrated in Figure 14 by overlaying the regression model for condition factor, lipid, weight and IGF-I for each year class examined. In both years the same overall pattern was seen.

The smolt-associated metabolic state is clearly a product of the developmental process of smolting. As seen in the present investigation, salmon smolts typically deplete liver glycogen, lipid, and lose condition factor (Hoar 1988). Due to limited plasma volume, we did not assess GH level, but increased plasma GH is characteristic of smolting under natural photoperiods (Bjornsson et al. 1989, 1995, McCormick et al. 1995). In this study we found that smolting chinook juveniles have high plasma IGF-I levels at the same time they exhibit depleted energy stores. In a laboratory study we also found that chinook salmon smolts in the spring have depleted glycogen stores while maintaining high plasma IGF-I levels (Beckman et al. 1998). It is unusual for animals to display both elevated GH and IGF-I. Elevated GH levels are characteristic of fasting animals (Farbridge and Leatherland 1992) and the high GH levels act to deplete lipid and glycogen while IGF-I levels are low (Duan and Plisetskaya 1993, Duan et al. 1995). In rapidly growing animals, GH levels are generally low, IGF-I levels are high, and lipid and glycogen levels are maintained or increase (Perez-Sanchez et al. 1995, Storebakken et al. 1991). Thus we may ascribe the smolt-associated metabolic state to the combination of high plasma GH and IGF-I, GH acting to deplete glycogen and lipid while IGF-I supports continued muscle and bone growth.

Smolting has often been viewed as a number of loosely organized endocrine, physiological, and behavioral events, which may not be directly interrelated. However, such a view may have evolved from an exclusive focus on laboratory or hatchery experiments where fish may not have received coordinated seasonal signals. In natural populations, spring-smolting salmonids respond to several seasonally coupled environmental signals (increasing photoperiod, temperature, food supply). Presumably, this response has been refined through selection to produce an adaptive process which allows juvenile salmonids to effectively make the freshwater to ocean transition typified by their life history. While acknowledging that smolting encompasses a broad number of physiological and behavioral changes, it is also evident that spring smolting occurs within a discrete seasonal period, suggesting that in naturally-rearing salmon the various components of smolting are temporally linked. This study's uniqueness lies in that it combined winter and spring sampling in a continuous series allowing us to observe a) the strongly catabolic state of wild chinook salmon in the winter b) the anabolic increase found in February - March prior to c) the strong depletion of energy reserves found during smoltification that appears to be accompanied by continued bone and muscle

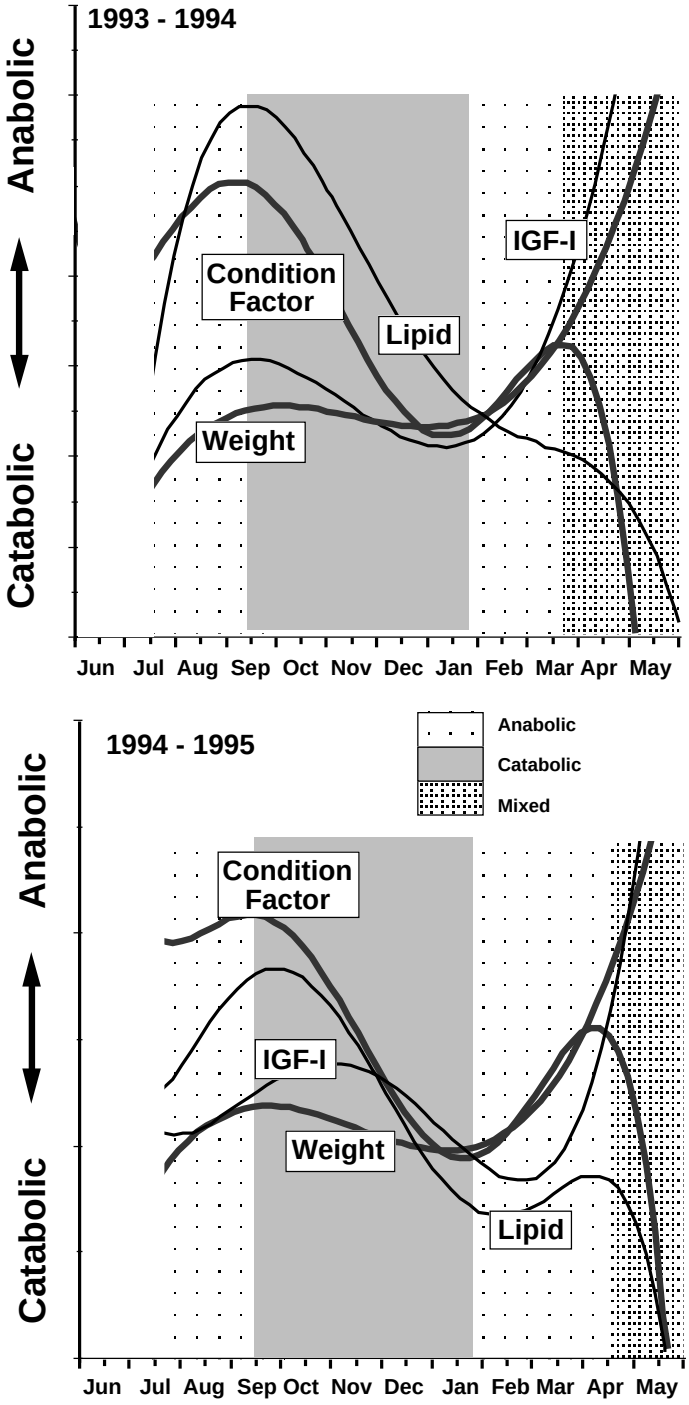

Figure 14. The seasonal anabolic-catabolic status of juvenile chinook salmon rearing in the Yakima River 1993 - 1994 (upper panel) and 1994 - 1995 (lower panel).

growth (smolt-associated metabolic state). These observations suggest that the smolting process may be an integrated product of a seasonal series of environmental stimuli and endocrine/physiological responses. Continued investigation into the relation of winter/spring feeding conditions, metabolic status, and growth may yield important results with regard to the control of smolting.

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CHAPTER 2

"DOES SMOLT SIZE REALLY MATTER?"

Publication Title A: The Relationship of Fish Size and Growth to Migratory Tendencies of Spring Chinook Salmon (*Oncorhynchus tshawytscha)* Smolts.

Brian R. Beckman[1], Donald A. Larsen[1], Beeda Lee-Pawlak[1],
and Walton W. Dickhoff[1,2]

Publication Title B. Insulin-Like Growth Factor-I and Environmental Modulation of Growth During Smoltification of Spring Chinook Salmon, (*Oncorhynchus tshawytscha*).

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A: The Relationship of Fish Size and Growth to Migratory Tendencies of Spring Chinook Salmon (*Oncorhynchus tshawytscha)* Smolts.

SUMMARY

We examined the relation of size and growth rate to downstream migration in yearling spring chinook salmon *Oncorhynchus tshawytscha*. A group of juvenile chinook salmon was graded by size into small and large categories: half the fish in each category were reared at an elevated temperature beginning in mid-February, resulting in four distinct treatment groups: Large Warm (LW), Large Cool (LC), Small Warm (SW), and Small Cool (SC). Fish from warm-water treatment groups displayed significantly higher growth rates through the spring compared to cool-water groups. Fish were released into a natural creek on two dates (25 March (Release 1) and 12 April (Release 2)) and downstream movement was monitored. For each release, fish that migrated past a weir within the first 5 d post-release had significantly higher spring growth rates than fish that did not migrate within that period. A similar comparison of release length to migration demonstrated significant differences only in Release 2. Also for Release 2, fish from the LW and SW treatment groups were recovered in higher proportions than fish from LC and SC groups. These results indicate that fish with relatively higher spring growth rates moved downstream sooner than fish with relatively lower growth rates. Furthermore, smolt size and migration were related with larger fish moving downstream sooner than smaller fish; however, this relation was weaker than that found for growth rate and migration.

INTRODUCTION

Rapid downstream movement and ocean entry may be valuable traits for juvenile salmonids released from hatcheries. A quick downstream migration decreases the time smolts are exposed to predation in riverine migration corridors and hastens their entry into relatively food-rich coastal waters (Raymond 1979). Several hundred million smolts are released each year from hatcheries in the Pacific Northwest; improving smolt migration is currently a major management goal (NMFS 1995). Smolt size has been suggested as a factor that can influence migratory behavior and subsequent survival to adulthood in juvenile salmonids. Large smolts have been found to migrate sooner than smaller fish in natural populations of coho *Oncorhynchus kisutch* and Atlantic salmon *Salmo salar* (Irvine and Ward 1989; Bohlin et al. 1993). Several studies have also shown that larger steelhead trout *O. mykiss* smolts within a year class tend to survive to adult at a higher rate than smaller fish (Ward and Slaney 1988; Ward et al. 1989; Henderson and Cass 1991). Similarly, large hatchery chinook *O. tshawytscha* and Atlantic salmon smolts migrate sooner than smaller fish (Ewing et al. 1984; Hansen and Jonsson 1985) and large hatchery coho and chinook salmon have relatively greater survival to adult (Bilton et al. 1982; Bilton 1984; Martin and Wertheimer 1989). In experimental studies, size categories may be created by manipulating growth rates of experimental fish in the last few months prior to release. Thus, large smolts may also be faster growing, so that differences in behavior and survival of large and small fish could be due to growth rate, body size, or both factors.

A few studies have noted that growth rate may have an influence on smoltification independent from body size. Wagner et al. (1969) noted that fall chinook salmon exhibiting high growth rates showed better seawater tolerance than larger, slower growing fish. Thorpe (1989) and Thorpe et al. (1989) showed that increased summer growth opportunity of Atlantic salmon parr increased subsequent percentage of fish undergoing smoltification at age 1. Okland et al. (1993) suggested that age at smolting in Atlantic salmon was inversely dependent on growth rate (with faster growing fish smolting at an earlier age) and rejected the hypothesis that a threshold size regulates smolting. Finally, Dickhoff et al. (1995) showed a relation between spring growth rate of hatchery spring chinook salmon juveniles prior to release and hatchery return of adults. Together these results led us to question whether past demonstrations of superior performance by large smolts were due to their physical size, or to the recent growth history of these fish, with fast growth leading to intensified physiological development and migratory performance.

This study was undertaken to evaluate the influence of fish body size and growth rate on the parr-smolt transformation of yearling chinook salmon. Differences in smoltification between juvenile chinook salmon of different size and from different spring growth regimes were assessed by downstream migration. Specifically, we tested the hypothesis that relatively faster growing fish, upon release into a natural creek, would migrate downstream sooner than relatively slower growing fish.

MATERIALS AND METHODS

Ten adult chinook salmon (5 male and 5 female) were obtained from spawning areas of the Yakima River in Washington State. Gametes were stripped from adults at the capture site and transported to a research hatchery at the Northwest Fisheries Science Center in Seattle. Eggs and alevins were incubated in Heath trays. Fish were reared with a seasonally changing water temperature and photoperiod (day length adjusted weekly to that of Seattle latitude 48^{o} N). In August 1993, 1,000 large (mean = 83 mm, SE = 0.4) and 1,000 small (mean = 60 mm, SE = 0.6) individuals were selected from 20,000 sub-yearling fish. Fish were maintained in four 1.3 m diameter circular tanks at 500 fish/tank with a flow of 10 l min.$^{-1}$. Maximum density was reached in March (LW tank) and was less than 18 g fish liter $^{-1}$. In March the density of the SC tank was 10 g fish l^{-1}. These densities correspond to density indices of 0.22 and 0.14. In January 1994 individual fish were weighed and measured, then passive integrated transponder tags (PIT tags, Destron) were implanted into the intra-peritoneal cavity. In mid-February temperature treatments were initiated, one tank containing fish from each size category received ambient water (7^{o}C) and one tank containing fish from each size category received ambient water heated to 11^{o} C. This resulted in four distinct experimental groups: small cool-water (SC), small warm-water (SW), large cool-water (LC), and large warm-water (LW). Temperatures in the different treatments converged by mid-April, when ambient water reached 11^{o}C and heated water was 12^{o}C. All fish were fed at a rate of 1.4% body weight/day (Biodiet Grower, Bioproducts Inc., Warrenton, Oregon) until mid-February. Feeding rates in the LW and SW groups were increased simultaneously with the change in water temperature to 1.8% body weight/day and were maintained throughout the remainder of the experiment.

Migration was tested on 25 March and 12 April 1994. Tests were conducted at Cooke Creek, a third order tributary of the Yakima River. Several days prior to release, test fish were removed from their rearing tanks (about 100 fish from each group (~ 400 total)), weighed and measured, then placed in a common holding tank. Water temperature in this tank was decreased from 8^{o}C to 5^{o}C over the course of three days. Fish from all groups were placed together into a 1,000-L oxygenated transport tank for transfer to the test site by truck. Water in the transport tank was tempered with dechlorinated ice to maintain a water temperature of 5 - 6^{o}C. Transfer from rearing site to release site took less than 3 h. Water temperature at each release was 5^{o}C. Fish were released into the creek soon after arrival, and monitoring of movement began immediately. In addition, 40 fish were randomly netted from the transport tank and placed into two 40 l plastic barrels placed into the creek (20 fish/barrel). The fish from the barrels were sampled after two days in order to characterize effects of transport and creek residence on physiological characteristics (data not reported). Transport stress and creek residence may have had some negative effects on fish, as 2 of the 40 fish died (Release 1, not attempted Release 2). Indigenous fish in Cooke Creek included small rainbow trout and sculpin *Cottus* spp. Irrigation diversions in the lower reaches of the creek prevented upstream passage of anadromous fish. Observations by snorkel-equipped divers indicated relatively little behavioral interaction between the released chinook salmon smolts and the indigenous fishes (G. McMichael, personal communication, Washington Department of Fish and Wildlife, Ellensburg, WA).

One PIT-tag detector (Biomark Inc. Boise, ID) was placed at the apex of each of two separate, V-shaped weirs located 1.0 (Weir 1) and 1.3 km (Weir 2) downstream from the release site. Cooke Creek has a moderate gradient with many riffle-pool sequences between the release site and Weir 1, including several large pools behind debris jams. Several riffle-pool sequences were also present between Weir 1 and Weir 2. Weirs consisted of 6.4 mm mesh hardware cloth stapled over 2.5 m by 1.1 m wood frames. Fish were detected as they passed through a 15 cm PVC pipe, the only downstream path through the weir. The time and date of passage for each fish was logged by the PIT-tag detector as fish passed downstream. At the second weir, fish were routed into a plywood holding box, which ensured that all fish were logged with 100% efficiency. Batteries and floppy discs in each PIT-tag detector were exchanged daily. Relative changes in flow were estimated by measuring creek height daily at 1700 hours. The creek was surveyed daily, from the release site to Weir 2, by walking down the creek in waders, 11 dead release fish were found 25 - 31 March.

Cooke Creek lies at the base of the Wenatchee Mountains. Extensive snow fields on the flanks of this range are subject to rapid melting on warm spring days, and such conditions occurred approximately a week after each release, resulting in flooding which allowed fish to bypass the weirs without detection. Flooding was a stochastic event that separated released fish into two groups: early migrating (EM), which passed weirs before flooding and were detected, and non-detected (ND), which were assumed to have passed weirs after flooding and were not detected.

A 300 m portion of the reach between the release site and Weir 1 was electrofished 1 week after the weir flooded out the second time to check for ND fish; this section was selected as it had a high density of fish-holding habitat. One study fish, along with 8 rainbow trout (which were much less abundant than the study fish upon initial release, G. McMichael, personal communication), was collected, indicating that few study fish remained. No large fish-eating birds or aquatic predators were seen near the creek during the experiment, though predation by aquatic mammals might have occurred. These observations suggested that fish not detected at a weir were not lost from the creek due to predation. Rather, fish that were released but not detected at a weir likely passed down the creek after the weir collapsed.

One to three days prior to release individual fish were weighed and measured. Instantaneous growth rates for each fish were calculated by: $(\ln L2 - \ln L1)*(T2-T1)^{-1}*100$ (where L1 = length at tagging, L2 = length at release, T2-T1 = days between release and tagging). Differences in release length and spring growth rate between groups were determined by one-way ANOVA, followed by Fisher's protected least significant difference test, differences were considered significant at $P = 0.05$ (Zar 1984). The relative spring growth rates and release length of EM and ND fish were similarly assessed with one-way ANOVA. Differences in the number of fish from each group (LW, LC, SW, SC) which were detected at the weir prior to flooding, were tested by chi square. Relations between release length or spring growth rate to passage time (hours from release to a Weir 1) were examined by Spearman rank correlations. All analysis were conducted with Statview II (Brainpower Inc., Cupertino CA).

RESULTS

Significant differences in length among treatment groups were present at release (Figure 1). For Release 1, LW fish were significantly larger than LC fish, which in turn were larger than both SW and SC groups, which were not significantly different from each other. Average lengths of each of the treatment groups for Release 2 were significantly different.

For Release 1, the LW fish grew at a significantly higher rate than the SW fish, which grew at significantly higher rate than either of the cool water groups (SC and LC, Figure 2). There was no significant difference between the SC and LC fish in growth rate. Both warm water groups (SW and LW) showed significantly higher growth rates than the cool water groups (SC and LC) for Release 2. There was no significant difference in growth rate between groups reared at the same temperature.

Of the 482 fish in Release 1, 150 were detected in the 6 days prior to the flooding which collapsed the weir (Figure 3). An additional 19 fish were detected from Release 1 after the weir was reinstalled. These 19 fish are designated as Late Migrants (LM) and are only considered in results when specifically mentioned. In the 5 days of monitoring conducted for Release 2, 170 of 324 fish released were detected.

Fish from Release 1 were first detected on the second day after release (Figure 3a). Peak migration past the weirs occurred on the third evening post release. The majority of fish moved past the weirs between mid-afternoon and midnight. Mid-afternoon movement began as the sun passed behind canyon walls, shading the creek. Fish from Release 2 began moving past the first weir almost immediately after release, with peak migration past the first weir occurring during the first evening post release (Figure 3b). Again, passage through the weirs occurred in the early evening hours. No relation between daily fish passage and water level in the creek was obvious for either release (Figure 3a, 3b).

We released four groups of fish on two different dates and monitored movement past weirs downstream of the release site. The data obtained could be evaluated in several different manners: comparing passage of individuals from different groups in succeeding blocks of time (relatively more individuals from high growth groups should pass a weir in earlier time blocks than in later time blocks) or comparing passage time (hours taken to move from the release site past a weir) of individuals from the different groups within a block of time (average passage time of high growth groups should be less than that of low growth groups).

There was no significant difference in the proportion of individual fish detected from different treatment groups in the block of time prior to weir collapse for Release 1 (Figure 4). A significant difference was found for Release 2. Fish from both warm water groups (SW and LW) were detected in numbers greater than expected, whereas fish from the cool water groups (SC and LC) were detected in numbers lower than expected.

Since PIT tags are individually coded, one may investigate the relations between size, growth, and whether or not individual fish were detected passing a weir. For Release 1, there was no significant difference in length between EM, ND or LM fish when they were liberated into the creek (Figure 5). For Release 2, EM fish were significantly larger than ND fish at liberation. Calculation of individual growth rates revealed that for each release, EM fish had significantly higher average spring growth

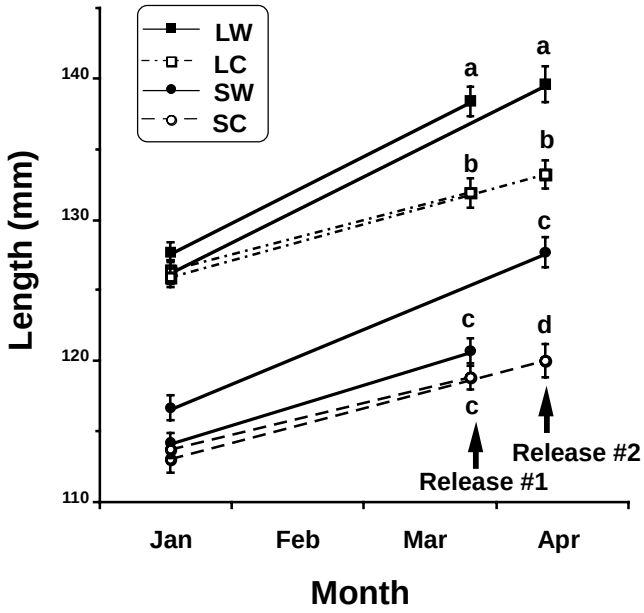

Figure 1. Mean fork length of juvenile chinook salmon from different treatment groups (LW = large-warm, LC = large-cool, SW = small-warm, SC = small-cool), at tagging in January and at 1 to 3 days before Release 1 (24 March) or Release 2 (10 April). Symbols indicate means; bars indicate standard error. For each release date, symbols with different letters are significantly different (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

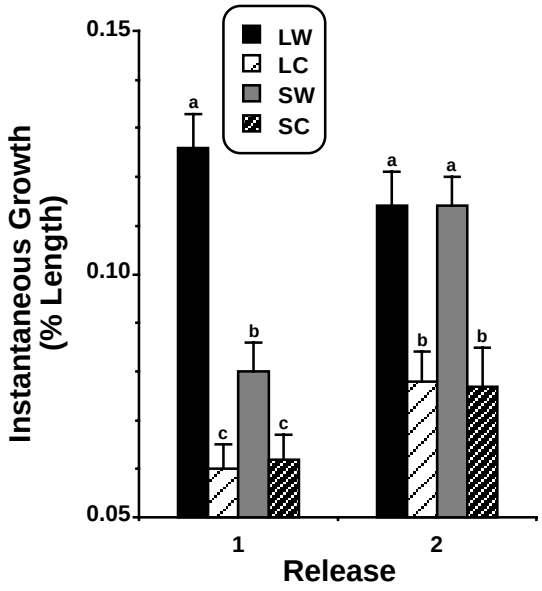

Figure 2. Mean instantaneous growth rates (% length) of juvenile chinook salmon from treatment groups (LW = large-warm, LC = large-cool, SW = small-warm, SC = small-cool), determined from 16 January to 24 March (Release 1) or from 16 January to 10 April (Release 2). Columns indicate means; bars indicate standard error. For each release date columns with different letters were significantly different (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

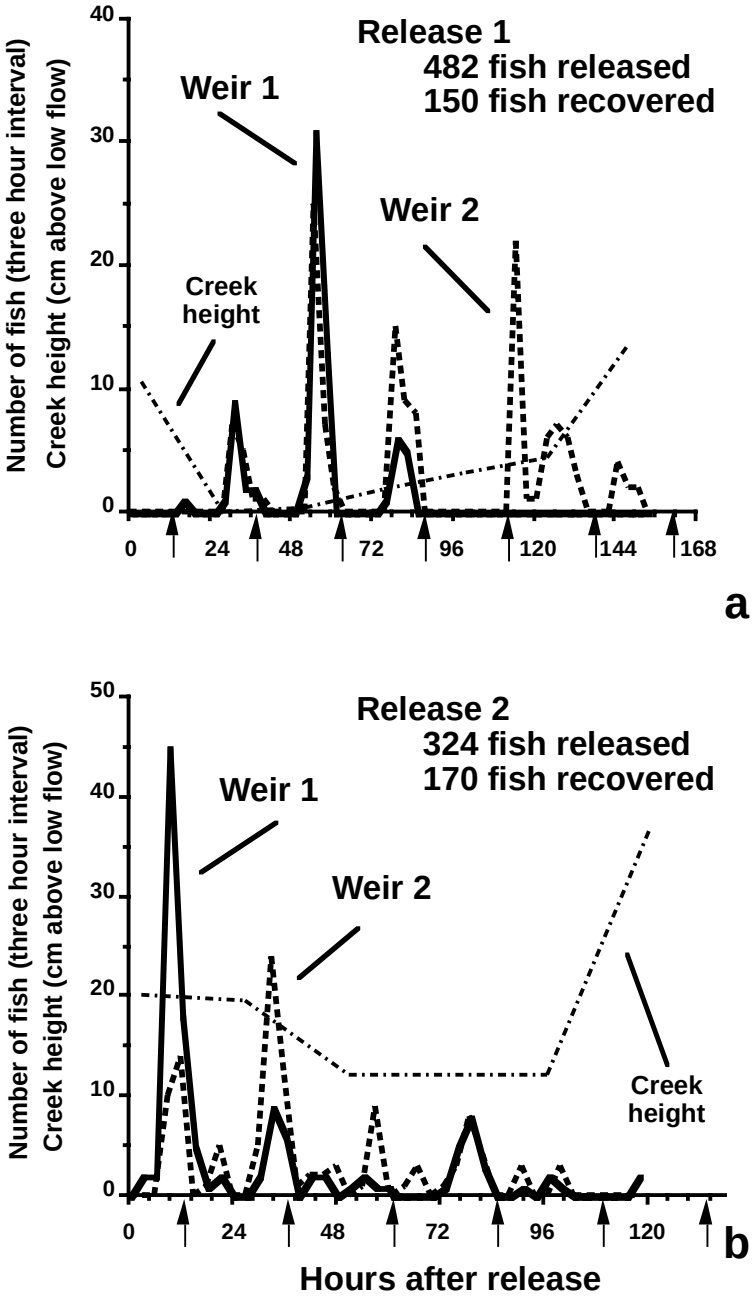

Figure 3. Temporal pattern of fish movement through two weirs on Cooke Creek. Release 1 (a) occurred at 1200 hours 25 March; Release 2 (b) occurred at 1300 hours 12 April. Fish detected at Weir 1 indicated by solid line, fish detected at Weir 2 indicated by dashed line. Daily creek height indicated by thin solid line (cm above low flow). Arrows below abscissa indicate midnight.

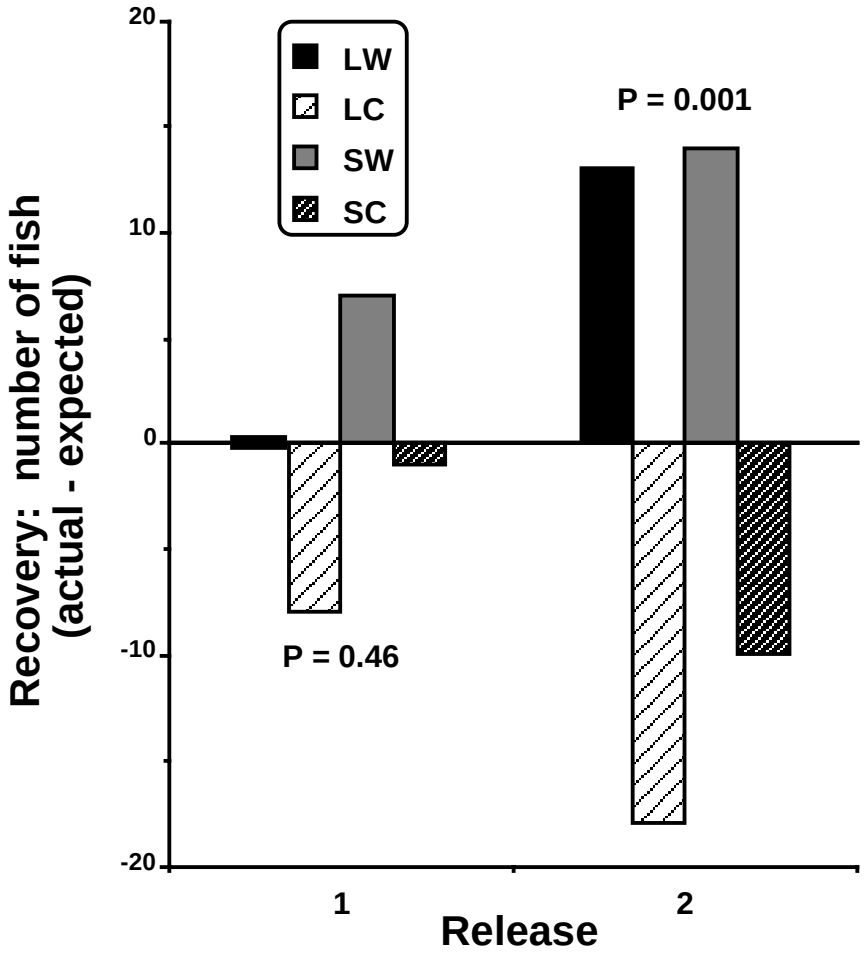

Figure 4. Recovery of fish from separate treatment groups for Release 1 and Release 2; LW = Large Warm, LC = Large Cool, SW = Small Warm, SC = Small Cool. Actual = total number of fish from given treatment detected at either weir. Expected = number of fish from a treatment released into creek multiplied by recovery proportion for all four treatments combined. P-values were calculated by Chi-square.

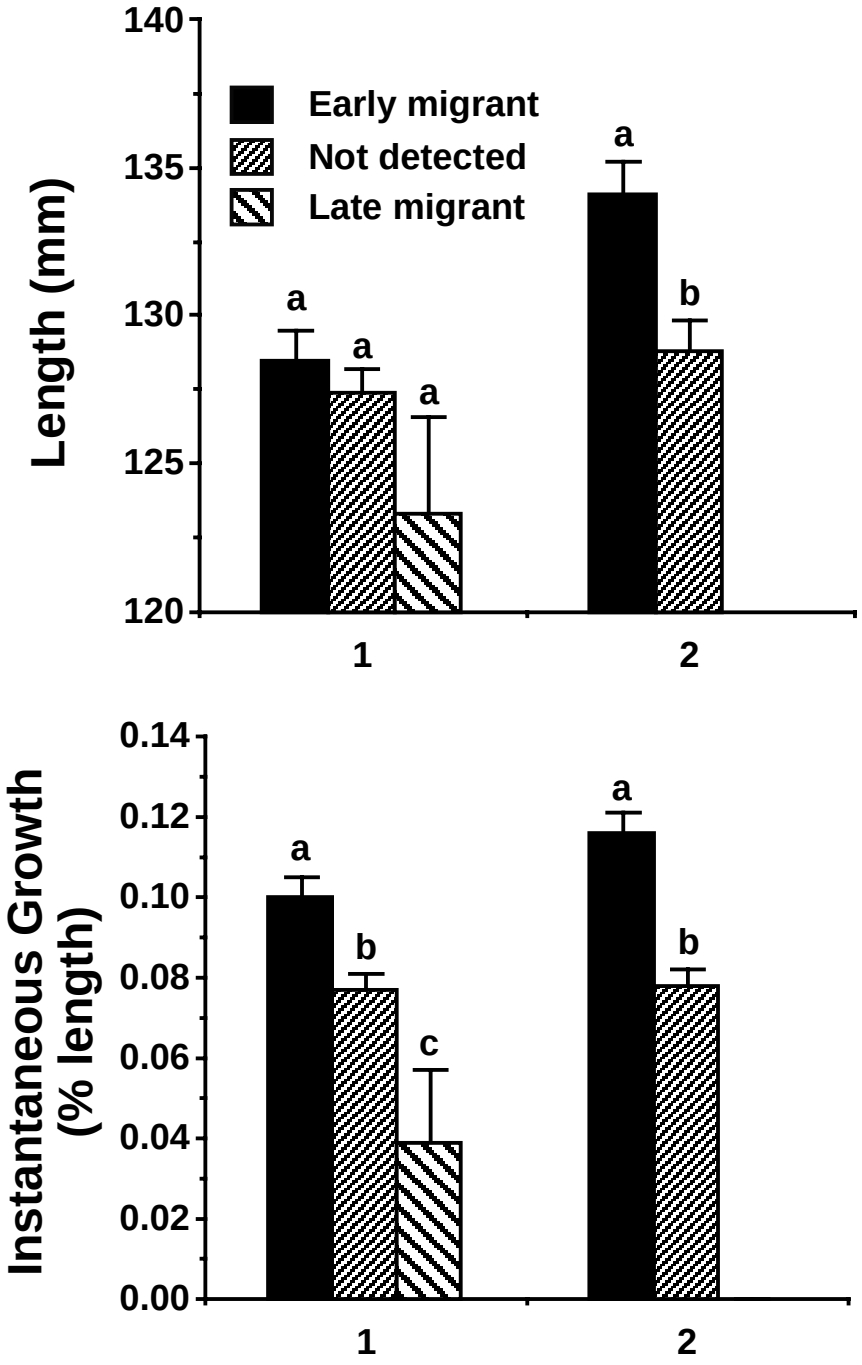

Figure 5. Mean lengths (24 March - Release 1; 10 April - Release 2) and instantaneous growth rates (16 January to 24 March - Release 1; 16 January to 10 April - Release 2), of fish released into Cooke Creek. For Release 1, early migrants detected 25 to 31 March, late migrants detected 5 to 17 April. For Release 2, early migrants detected 10 to 17 April. Columns indicate means; bars indicate standard error. For each release date columns with different letters were significantly different (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

rates than ND fish (Figure 5). In addition, for Release 1, LM fish had the lowest growth rates pre-release, significantly lower than both ND and EM fish.

The possible relation between relative size or growth rate and passage time (hours from release to weir detection) for fish detected at Weir 1 was also examined. No significant relation was found for Release 1 (length vs. hours to W1; growth vs. hours to W1). For Release 2, length was not related to passage time while growth was (ρ = -0.301). Thus we found an indication that relatively faster growing fish displayed a shorter passage time from the release site to Weir 1 within the 5 day time block that the weir was intact after Release 2.

DISCUSSION

Juvenile salmonids show a striking urge to move downstream to the ocean during specific seasonal windows and the initiation of this behavior is an integral part of the smoltification process (Hoar 1976). We hypothesized that relatively high growth rate during the spring would lead to an earlier or more complete initiation of the smoltification process for fish held in a hatchery (Dickhoff et al. 1995, 1997; Beckman et al. in press). In turn, we hypothesized that individuals from high growth groups, because they were in an advanced state of smoltification, would display a greater propensity to move downstream when released into a natural creek (more fish from high growth groups would have undergone the behavioral transition which resulted in them displaying downstream movement). To be specific: individuals from high growth groups would move downstream sooner than individuals from low growth groups.

Three analyses supported our hypothesis: (1) average growth rates of early migrating fish were greater than those of non-detected fish in both releases; (2) for Release 2, we recovered a higher proportion of fish from fast growing groups than from slow growing groups. In addition, (3) faster growing fish showed a decreased passage time in Release 2. We also found significant relations among fish size and migration, larger fish tended to migrate sooner than smaller fish, especially for Release 2. However, for Release 1, large fish were not observed at a weir in greater numbers than small fish. In Release 2, small fast-growing fish showed decreased passage time as compared to large slow-growing fish, as they were caught at higher relative proportions at the weir. Overall, our results do not strongly discriminate between size and growth rate as factors related to migratory performance; yet, our results represent the first positive test of the hypothesis that juvenile chinook salmon reared at relatively high spring growth rates show an increased migratory disposition upon release.

Observations of wild smolts at downstream counting weirs have suggested that large fish may migrate downstream sooner than smaller fish (Irvine and Ward 1989; Burgner 1991; Bohlin et al. 1993; Wood et al. 1993). A similar observation has also been made with releases of experimental or hatchery fish (Ewing et al. 1984; Hansen and Jonsson 1985). These results have led to the conclusion that large fish show increased migratory dispositions sooner than do small fish. Our results do not conflict with these observations, size and growth rate are inevitably related - faster growing fish become larger than slower growing fish over the same time interval. However, our results might suggest caution with regard to a cause and effect relation between fish size and migratory behavior. Our results are not conclusive enough to challenge the established paradigm

which relates smolt size to migratory performance and subsequent survival to adult. We report on only two releases from one unreplicated growth experiment, conducted in a small creek over a short distance, with several obvious difficulties with regard to monitoring fish. However, these results are interesting enough to stimulate further experiments similar to the one reported here - such that one may discriminate between the relative effects of growth and size on smoltification. Indeed, our study re-enforces the work of Wagner et al. (1969), Thorpe (1989), Thorpe et al. (1989), Okland et al. (1993) and Dickhoff et al. (1995) who all suggest that relatively higher growth rate may influence timing of the smoltification process.

No readily apparent, intuitive relationship between growth rate and downstream migratory disposition exists in juvenile salmonids. However, several studies have shown that physiological smolt development and development of downstream migratory tendencies are correlated. Zaugg and Wagner (1973) showed that an advanced photoperiod in the spring induced accelerated smolt development (as measured by increases in gill Na^+-K^+ ATPase activity) in steelhead trout. The physiological advance was accompanied by an earlier occurrence of downstream movement by advanced fish in an experimental channel. Hart et al. (1981) found hatchery chinook salmon with higher Na^+-K^+ ATPase activities migrated out of a rearing channel sooner than fish with lower Na^+-K^+ ATPase activities. Muir et al. (1994) showed that advanced photoperiod and increased rearing temperatures accelerated smoltification and decreased downstream passage time in hatchery-reared spring chinook salmon. These three studies provide no direct evidence of a coupling of physiological change and migrational behavior. They do suggest, however, that physiological change and migrational behavior are tightly linked temporally. Moreover, they indicate that environmental manipulations that affect physiological development also change migratory behavior.

Hormonal secretions can alter or stimulate behavioral activities of many vertebrates (Becker et al. 1992). Smoltification has excited much interest among endocrinologists, as there are striking changes in thyroid hormone, cortisol, insulin, and growth hormone plasma levels during this period (Dickhoff et al. 1990). The occurrence of these hormonal fluctuations during smoltification has led to the postulate that one or a number of these hormones are responsible for the change in migratory disposition (Grau et al. 1981; Iwata 1995). Beckman et al. (in press) found no difference in plasma levels of thyroxine between fish from fast- and slow-growing groups reared in an identical manner as those in this experiment. However, they did find consistent differences in plasma insulin-like growth factor-1 (IGF-1) between fast- and slow-growing fish during March and April. Insulin-like growth factor-1 is strongly linked to control of somatic growth in vertebrates (Daughaday and Rotwein 1989), though it has never been associated with migratory behavior. Considering the relations between growth, growth hormone (GH), IGF-1 and smoltification (Dickhoff et al. 1997), it might be appropriate to more closely examine the effects of GH/IGF-1 on migratory behavior in juvenile salmonids.

This study was not designed to simulate natural movement of smolts out of a stream reach; rather it was designed to measure differences in migration between different treatment groups. Transportation stress and crowding, followed by introduction to a novel habitat, may have influenced fish movement. However, stress was equally applied to all groups since they were transported and released together. The release and

weir sites were selected such that there were a number of pools upstream from the first weir capable of holding fish. In this manner, we hoped that stress-induced movement would occur upstream from our monitoring site, leaving only smolt-related movement to be measured at the weir. Even if stress-related movement past a weir did occur, there is no obvious reason to suggest fish from different treatments should have been differentially stressed upon release.

Since downstream movement was linked to release characteristics of smolts, as hypothesized, we contend that we were able to discriminate downstream movement associated with smoltification. This assertion is strengthened by the observation that downstream movement was faster and more prevalent in fish of Release 2, which may have been closer to their peak of smolt development. We found peak Na^+ K^+ ATPase values for fish in a companion study in mid-April (Beckman et al. in press). Displays of downstream movement would be expected to be heightened later during the smolting period coincident with peak Na^+ K^+ ATPase activities (Zaugg 1981). Our two tests of migration were not replicates. Rather, they were conducted at different points in relation to smolt development; accordingly, different results might be expected.

Temperature absolutely controls maximum growth rate in fishes (Brett 1979). Holtby et al. (1989) showed a strong positive relation between interannual spring stream temperature and median migration timing of coho salmon smolts. Relatively higher spring stream temperatures would result in higher potential growth rates in juvenile salmonids. Based on our results, the higher growth rate could result in the earlier recruitment of the endocrine mechanisms which lead to downstream migratory behavior.

This work suggests that hatchery rearing practices should be examined in regard to the growth rates of fish prior to release. Elevated growth prior to release could lead to increased migratory performance of smolts. Temperature was utilized in this study to elevate growth. Whether simple increases in ration, at a constant temperature, would also have a stimulatory effect is unclear, especially as many hatcheries already feed rations designed to produce optimal growth. A larger scale test of the relative effects of water temperature and feeding rate and their subsequent effects on growth and smolt performance is warranted.

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B: Insulin-like Growth Factor-I and Environmental Modulation of Growth during Smoltification of Spring Chinook Salmon (*Oncorhynchus tshawytscha)*.

SUMMARY

The relations among rearing environment, fish size, insulin-like growth factor-I and smoltification were examined in yearling spring chinook salmon (*Oncorhynchus tshawytscha)*. Juvenile chinook salmon were size-graded into small and large categories. Half of the fish in each group were reared at an increased temperature and feeding rate beginning in mid-February, resulting in four distinct treatment groups: large warm-water (LW), large cool-water (LC), small warm-water (SW), and small cool-water (SC). Increased temperature and feeding rate resulted in overall higher growth rates for the LW and SW groups. Temporal increases in insulin-like growth factor-I (IGF-I) were found in all groups through the spring. Plasma IGF-I levels were significantly higher in warm-water groups than cool-water groups from late March through May. Size itself appeared to have little relation to plasma IGF-I levels. Simple regression showed a significant relation between plasma IGF-I and growth ($P < 0.001$, $R^2 = 0.50$). No differences were found between treatment groups in other physiological parameters assessed (plasma thyroxine, gill Na^+-K^+ATPase, liver glycogen, body lipid).

INTRODUCTION

Environmental factors such as photoperiod, temperature and food supply modulate growth and development. A positive correlation between growth and development has been noted in a diverse array of animals: insects (Beck, 1971; Bradshaw and Johnson, 1995), amphibians (Wilbur and Collins, 1973), and fish (Policansky, 1983; Stearns, 1983). Juvenile salmonids offer an interesting model for the study of such processes as the growth hormone (GH) - insulin-like growth factor I (IGF-I) endocrine axis appears to have a dual role, integral to both smoltification and growth (Komourdjian et al., 1976b; Dickhoff et al., 1997).

Smoltification is a developmental process which results in juvenile salmonids undertaking a freshwater to seawater transition (Hoar, 1988). A number of hormones have been implicated in stimulating smoltification, including thyroid hormones, cortisol and GH (Dickhoff, 1993). In particular, GH has been shown to stimulate hypo-osmoregulatory ability (Komourdjian et al., 1976a; Clarke et al., 1977; Richman and Zaugg, 1987), including increased activity of Na^+-K^+ ATPase in the gill (Bolton et al., 1987; Collie et al., 1989). However, a role for IGF-I in smoltification is not yet clearly established. Rainbow trout (*O. mykiss*) injected with IGF-I showed enhanced seawater tolerance while IGF-I treatment in coho salmon (*O. kisutch*) resulted in increased Na^+-K^+ ATPase activity (McCormick et al., 1991; Madsen and Bern, 1993) and GH injections increase IGF-I mRNA in gill (Sakamoto and Hirano, 1993). Plasma levels of IGF-I increase in Atlantic salmon (*Salmo salar*) during smoltification (Lindahl et al., 1985) and IGF-I mRNA content also increases in gill and liver of coho salmon (Duguay et al., 1994).

We have chosen to approach the question of environmental control of the GH/IGF-I axis, growth and smoltification. We manipulated water temperature and feeding rate during the spring smolting period in order to examine the relation between growth, IGF-I and smoltification. Since the characteristics of plasma GH during smoltification are relatively well established (Sweeting et al., 1985; Björnsson et al., 1989; Boeuf et al., 1989; Young et al., 1989; Björnsson et al., 1995; McCormick et al., 1995), we chose to focus on plasma IGF-I levels. In order to separately evaluate and discriminate fish size and growth we graded our experimental population of chinook salmon (*O. tshawytscha*) into "small" and "'large" fish and modified environmental conditions for each size class. We thus produced a 2 x 2 bloc design with fish size and growth conditions the factors manipulated. This allows us to determine whether size has a physiological effect independent of growth rate. We measured growth rate and IGF-I, and four established physiological parameters of smoltification: plasma thyroxine (T4), gill Na^+- K^+ ATPase, liver glycogen and body lipid.

MATERIALS AND METHODS

Adult spring chinook salmon were captured near spawning areas in the Yakima River, a tributary of the Columbia River located in central Washington State. Gametes were stripped from adults at the capture site and transported to a research hatchery in Seattle. Eggs and alevins were incubated in Heath trays. Fry were transferred to 1.3 m

circular tanks and reared according to standard hatchery techniques (Piper et al., 1982). In August 1993, 20,000 subyearling fish were sorted into different size classes. Approximately 2,000 fish from both small (mean length = 60 mm; SE = 0.6) and large (mean length = 82.5 mm; SE = 0.4) size categories were retained. Fish from each size category were distributed into four tanks at 500 fish per tank (for a total of 4,000 fish in 8 tanks). Fish were reared with a seasonally changing water temperature and photoperiod (day length adjusted weekly to that of Seattle latitude 48^{o} N). Beginning in mid-February, water for two tanks from each size group was heated above ambient temperature (Fig. 1). This resulted in four treatment groups: large warm-water (LW), large cool-water (LC), small warm-water (SW), and small cool-water (SC), each contained in replicate tanks. All fish were fed at a rate of 1.4% body weight/day (Biodiet Grower, Bioproducts Inc., Warrenton OR) until mid-February. Feeding rates in the LW and SW groups were increased simultaneously with the change in water temperature to 1.8% body weight/day and maintained throughout the remainder of the experiment.

A monthly inventory of all tanks was made beginning on 20 January. Lengths and weights were measured from 60 fish per tank. Instantaneous growth rates (IG) for the period between inventories were calculated for each tank by:

$$IG = (\ln(\text{length 2} - \text{length 1}) \times (\text{day 2} - \text{day 1})^{-1}) \times 100,$$

where length 1 = mean length on day 1, length 2 = mean length on day 2, and day 2 - day 1 = number of days between measurements. Outliers (2 SD > mean) were removed from length and weight data before analyses were made.

Beginning in mid-January and continuing on a biweekly schedule through May, six fish were killed from each tank (12 fish/treatment) to obtain physiological samples. Twelve fish at a time were netted from tanks and placed in 20-L buckets. Fish were placed one at a time into a lethal concentration (0.2 g/L) of tricaine methanesulfonate (MS-222). Fish were weighed and measured, the tail was cut, and blood was collected in heparinized glass tubes from the caudal peduncle. Blood was centrifuged for 3 min at 3000 x g, and plasma was removed, frozen, and stored at -80^{o} C. Gill tissue was removed from three arches and placed in a solution of sucrose, EDTA, and imidazole according to methods described by Zaugg (1982), and then frozen on dry ice and stored at -80^{o} C. Livers were removed and immediately smashed and frozen between two pieces of dry ice. Liver chips were then placed in a well of a 24-well plate and stored at -80^{o} C. Fish carcasses were individually placed in plastic bags and stored frozen at -80^{o} C.

Fish in our experiment experienced mortality due to an epizootic of bacterial kidney disease (*Renibacterium salmoninarum*, BKD). Fish with obvious signs of BKD were not sampled. Furthermore, physiological data from fish that had low hematocrits (< 20%) were not included in any analysis.

Total plasma IGF-I concentration was determined according to Moriyama et al. (1994). Plasma T4 concentrations were determined according to Dickhoff et al. (1982). Gill Na^{+} K^{+} ATPase activities were measured using the method of Schrock et al. (1994). Liver glycogen content was determined with the method described by Plisetskaya et al. (1994). Whole body lipid was determined by the method of Soxhlet (AOAC, 1975) with lipid extracted with methylene chloride.

Data were first examined for differences between replicate tanks within treatments. Data for a treatment were pooled if there was no significant effect ($P > 0.05$)

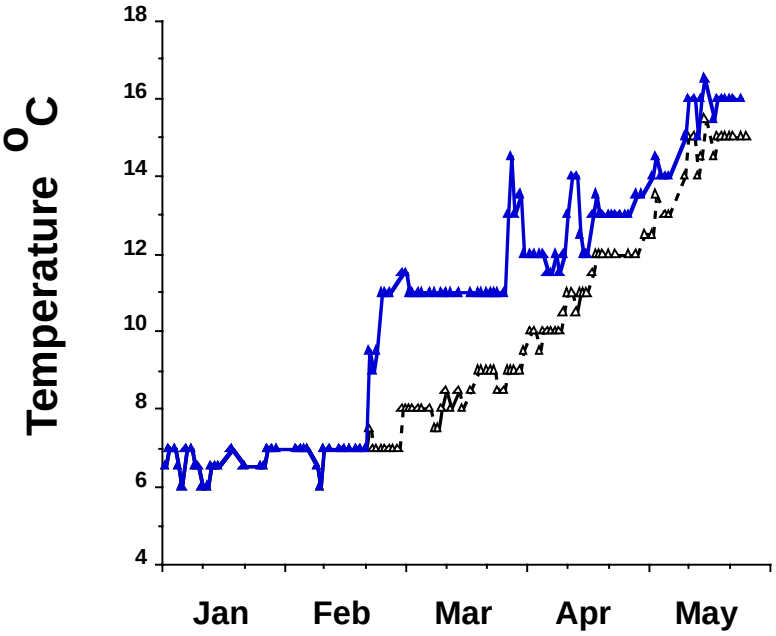

Fig. 1. Water temperatures of experimental groups; ambient (open triangles - dashed line) and heated (filled triangles and solid line).

of replication in a two-way analysis of variance (ANOVA) examining date and replicate. Results were then examined using a three-way ANOVA with date, temperature, and size being the effects modeled. If significant effects were found, differences between individual means (either differences between treatments for a given date or differences between dates for a given treatment) were examined using one-way ANOVA followed by Fisher's protected least significant difference (PLSD). Results were considered significant at $P < 0.05$. Significant differences between treatment groups for a given date are noted in the figures, significant seasonal differences within a treatment group are simply noted in the results. Linear regression was used to examine the relationship between plasma hormone levels and instantaneous growth. Mean plasma hormone level found in fish from a single tank, for a growth interval, were regressed against instantaneous growth for that interval. All statistical analyses were conducted using Statview II (BrainPower Inc., Cupertino, CA).

RESULTS

Significant differences in size between large and small treatment groups were found on all dates. All treatment groups increased in both length and weight from January to May (Fig. 2); growth was higher in groups maintained in the heated water (LW, SW) than for groups held at ambient temperature (LC, SC), as size diverged significantly between them. Significant differences in length and weight were found between replicate tanks in SW, SC, and LC treatment groups (Fig. 2). Over the course of

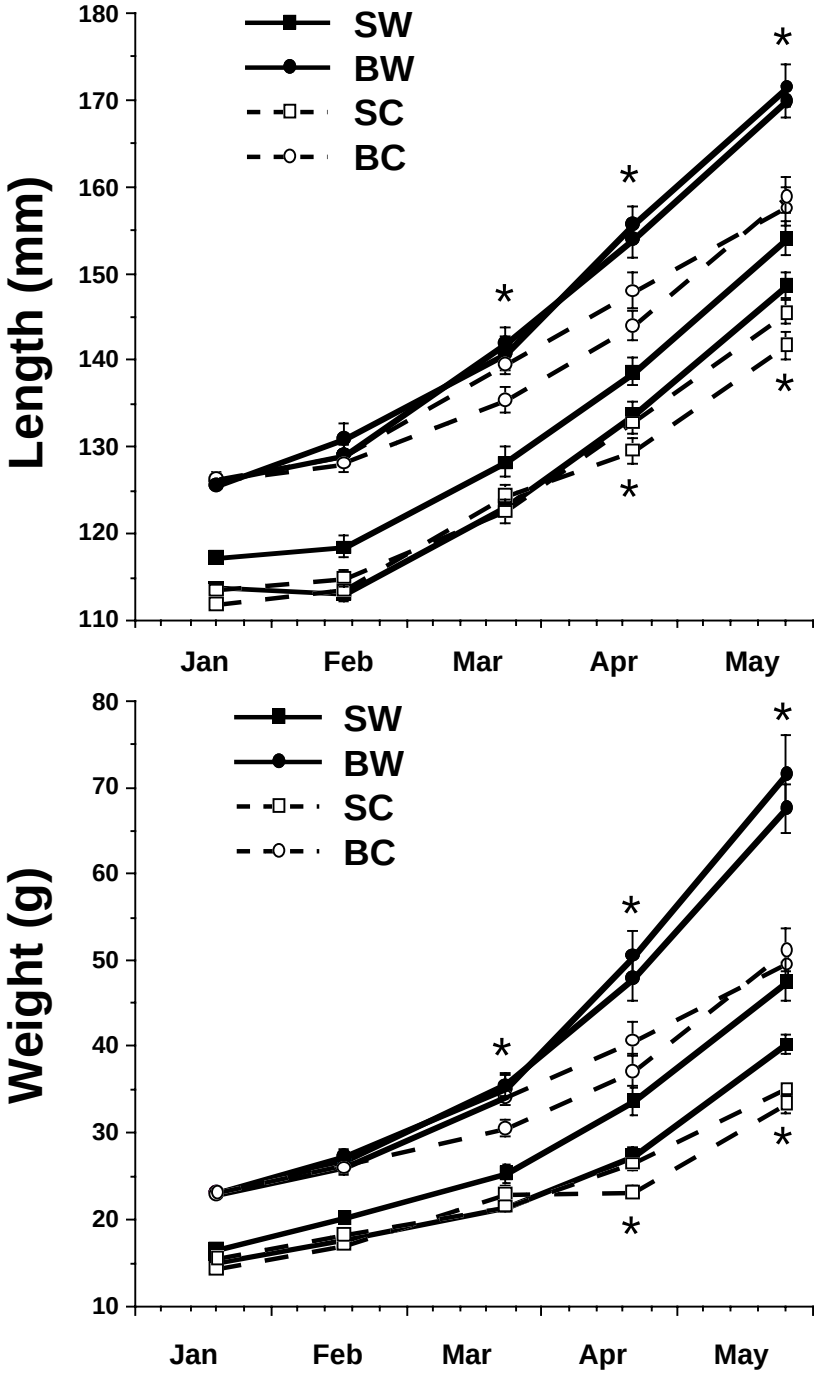

Fig. 2. Length (a) and weight (b) of replicate tanks of four treatment groups; small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). One replicate tank of the SW treatment was larger than other small size groups throughout the experiment. Asterisks indicate additional significant differences within size groups for a given date (LW vs. LC or SW vs. SC respectively, one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

the experiment, length increased from 125 to 158 mm for the LC groups and to 172 mm for the LW groups. The SC groups grew in length from 112 mm to 140 and 145 mm, and SW groups grew from 112 and 117 mm to 150 and 155 mm, respectively.

Size differences among large or small fish reared at different temperatures became apparent in March and April. For large fish, significant differences between tanks were found on 18 March, when fish from one of the LC replicates were smaller than fish from either LW tank or the second LC tank. Both LW replicates were significantly larger than both LC replicates in April and May. One of the SW replicates was significantly larger than the other small-fish groups throughout the study. Other differences became apparent on 20 April, when one of the replicates in the SC treatment was significantly lighter and shorter that the other SC tank and the SW groups. On 23 May both SW groups were significantly larger than the SC groups. A power analysis (Zar, 1984) for n = 60, $P = 0.05$, and power = 0.9 indicated that we could detect a significant difference as small as 0.5 mm between mean lengths of fish in different tanks.

Instantaneous growth (based on length) for treatment groups during four successive intervals, beginning in mid-January and ending in mid-May are shown in Fig. 3. Significant effects of date, temperature, and date by temperature in a three-way ANOVA suggest differences in growth, both seasonally and between treatments. Overall, a lack of statistical power (growth rates were established from average size data generated for each replicate tank of fish, therefore n = 2 for each treatment on a given date) precluded the ability to establish significant differences between individual treatments for any given time period. However, the statistically significant differences in length and weight, which became apparent in April, confirm that there were growth differences between warm and cool water treatment groups during March-April. Growth rate for all groups doubled from January-February to February-March, but there were no clear significant differences among the different experimental groups for the two periods. Growth rate for LW and SW increased again in March-April, whereas LC and SC remained similar to what was found for February-March. Growth rates for all groups were similar in the April-May period.

Plasma IGF-I increased significantly between late January and mid-February; values rose from about 70 to greater than 85 ng/ml (Fig. 4). Both SW and LW treatments showed a further significant increase in mid-March to values greater than 110 ng/ml. In mid-March significant differences between treatments were found and these differences were maintained through May, with IGF-I levels consistently higher in the warm-water groups. There was little apparent effect of size on plasma IGF-I level. There were no differences in IGF-I between LC and SC treatments. Fish from the LW treatment had significantly higher IGF-I than the SW fish in only two (late March and late May) of the nine dates measured. For all five physiological parameters examined, significant differences between replicate tanks within a treatment were only found for SC fish for body lipid. Accordingly, all replicate values were pooled within treatments.

Plasma thyroxine (T4) levels increased significantly from late February to May in all groups (Fig. 5). There were no significant differences between treatment groups on any one date.

Gill Na^+-K^+ ATPase activity increased significantly in all groups to reach peak values in late April (Fig. 6). The only significant difference between treatment groups

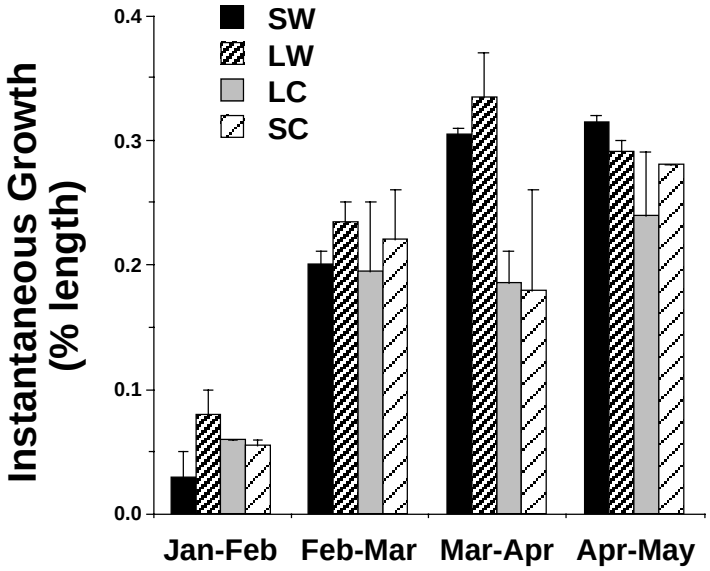

Fig. 3. Instantaneous growth for fish in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC).

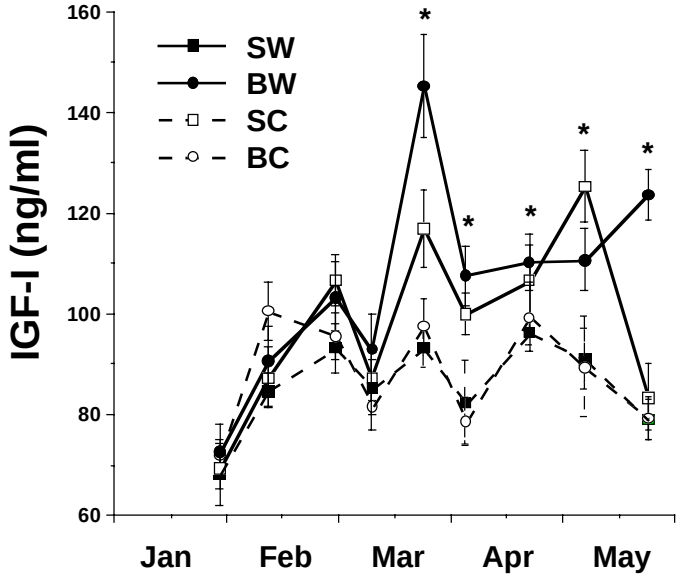

Fig. 4. Plasma insulin-like growth factor-I (IGF-I) concentrations in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisks indicate significant differences between treatment groups for a given date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

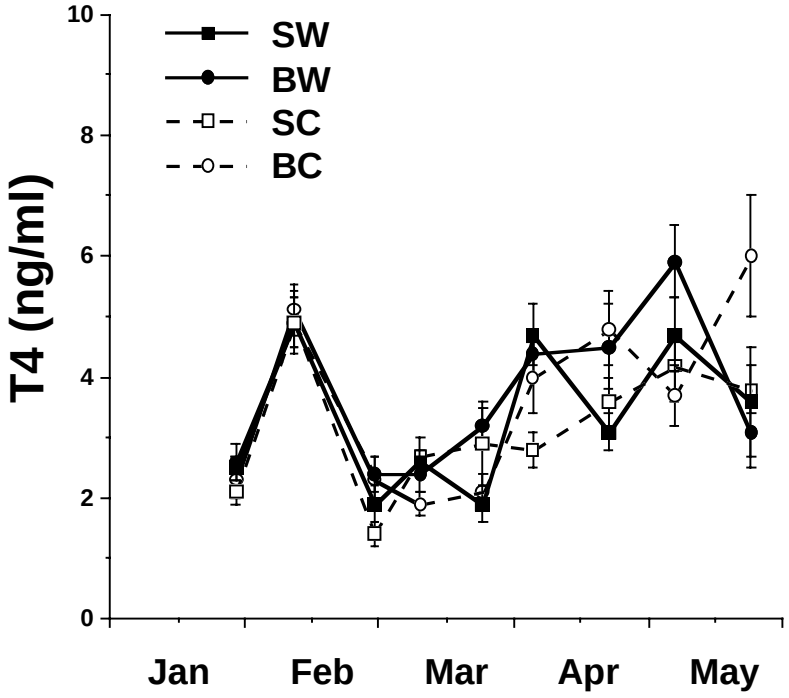

Fig. 5. Plasma thyroxine (T4) concentrations in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC).

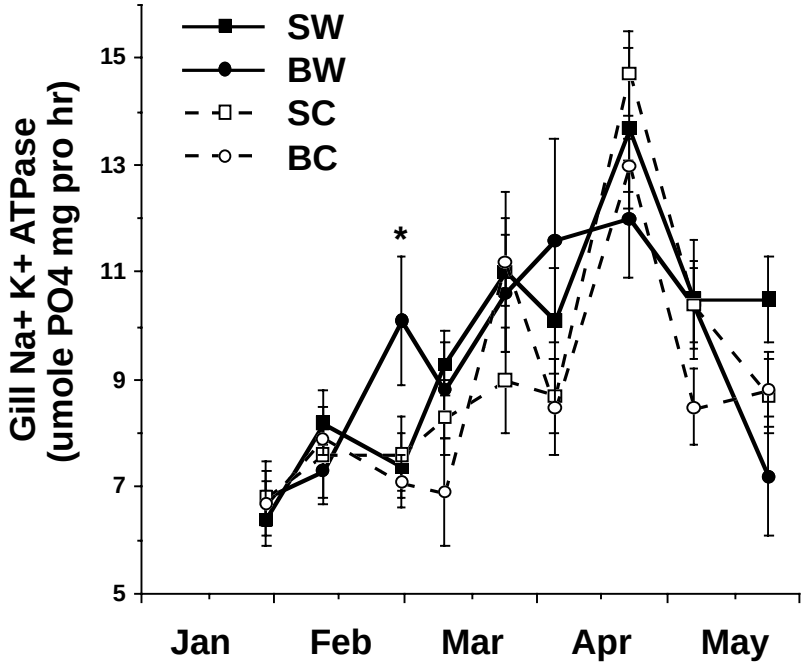

Fig. 6. Gill Na^+ K^+ ATPase activities in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisk indicates a significant difference between treatment groups for a given date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

was observed in late February, when the LW group had a higher value than the other groups.

Liver glycogen concentrations for all treatments were highest in January-February, declined significantly from February to the end of March, and then remained relatively low in April and May (Fig. 7). Significant differences between treatment groups were found on only one date in April, when glycogen values for LC fish were significantly lower than the other treatments.

Whole body lipid levels were relatively constant in most groups over time (Fig. 8). Only the LC group showed a significant decline from initial body lipid levels. In late February, LW fish had higher lipid levels than either cool-water group, and this was the only time when significant differences were found among treatment groups.

A highly significant ($P < 0.001$, $R^2 = 0.50$) positive relationship was found between mean IGF-I level in a tank during a growth interval and instantaneous change in length for that interval (Fig. 9). Two things should be noted about this relation: a) this relation was established by comparing average IGF-I values found during a growth period vs. growth and b) if one removes the January data a notably poorer relation is found ($P < 0.01$, $R^2 = 0.29$). A similar comparison of mean plasma T4 levels in a growth interval and instantaneous change in length showed a marginally non-significant relation ($P = 0.06$, $R^2 = 0.12$).

DISCUSSION

There are several recent reports on plasma IGF-I levels in juvenile salmonids (Moriyama et al., in press; Shearer et al., in press; Silverstein et al., submitted). These results are significant in two respects: a) the relation of IGF-I to somatic growth in juvenile salmon and b) a potential specific role of IGF-I in smoltification. In our results we found clear differences in IGF-I related to growth but these differences in IGF-I between treatment groups showed no relation to other physiological indices of smoltification.

The GH - IGF-I axis in salmonids appears to fit the somatemedin hypothesis of Daughaday et al. (1972) with GH promoting increased levels of IGF-I mRNA in the liver (Cao et al., 1989; Sakamoto and Hirano, 1993; Duan et al., 1994; Shamblott et al., 1995) and subsequent levels of IGF-I protein in the blood (Moriyama et al., 1994; Moriyama, 1995). Injection of IGF-I stimulates growth in coho salmon (McCormick et al., 1992) and starvation reduces hepatic production of IGF-I like activity in trout (Komourdjian and Idler, 1978) and hepatic IGF-I mRNA levels in coho salmon (Duan et al., 1995). In addition, feeding and fasting and manipulation of growth rates by ration and protein intake results in a good correlation between circulating IGF-I and growth rate in another teleost, the gilthead seabream (*Sparus aurata*) (Pérez-Sánchez et al., 1994, 1995).

The relation of IGF-I and growth is of great interest; however, the relation may vary depending on either the season in which the relation is examined or the statistical relation used. In our study, fish held at relatively warm water temperature and fed a correspondingly higher level of feed, showed elevated levels of IGF-I. Regression analysis suggested that much of the seasonal variation in growth within our treatment groups was explained by differences in IGF-I during each growth interval. However, it must be emphasized that we analyzed average IGF-I levels during one-month growth

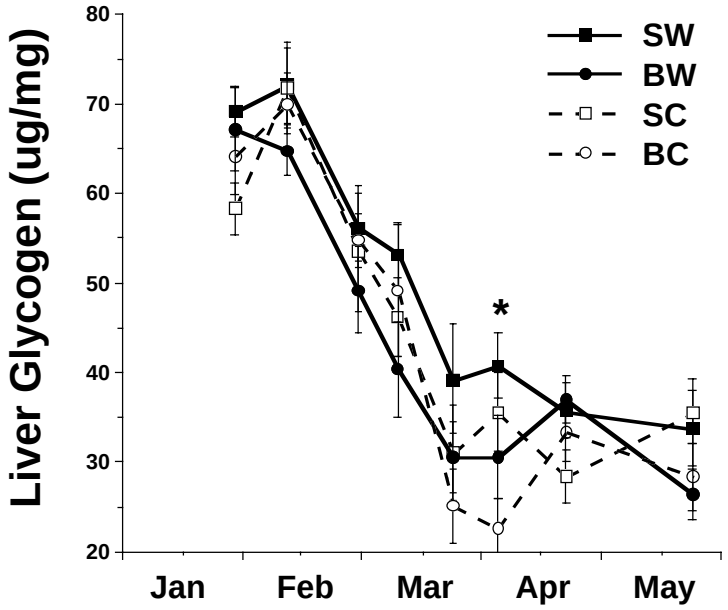

Fig. 7. Liver glycogen levels in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisk indicates a significant difference between treatment groups for a given date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

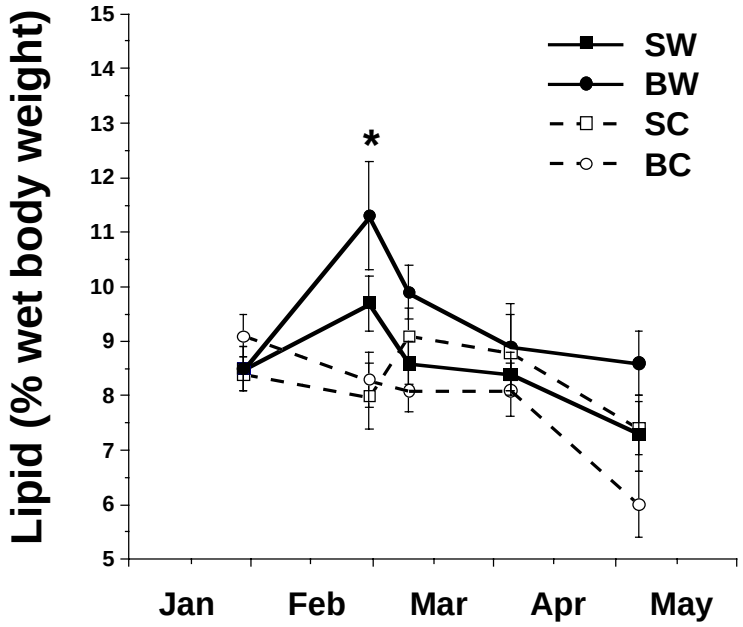

Fig. 8. Whole body lipid (% wet weight) in four treatment groups, small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisk indicates a significant difference between treatment groups for a given date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

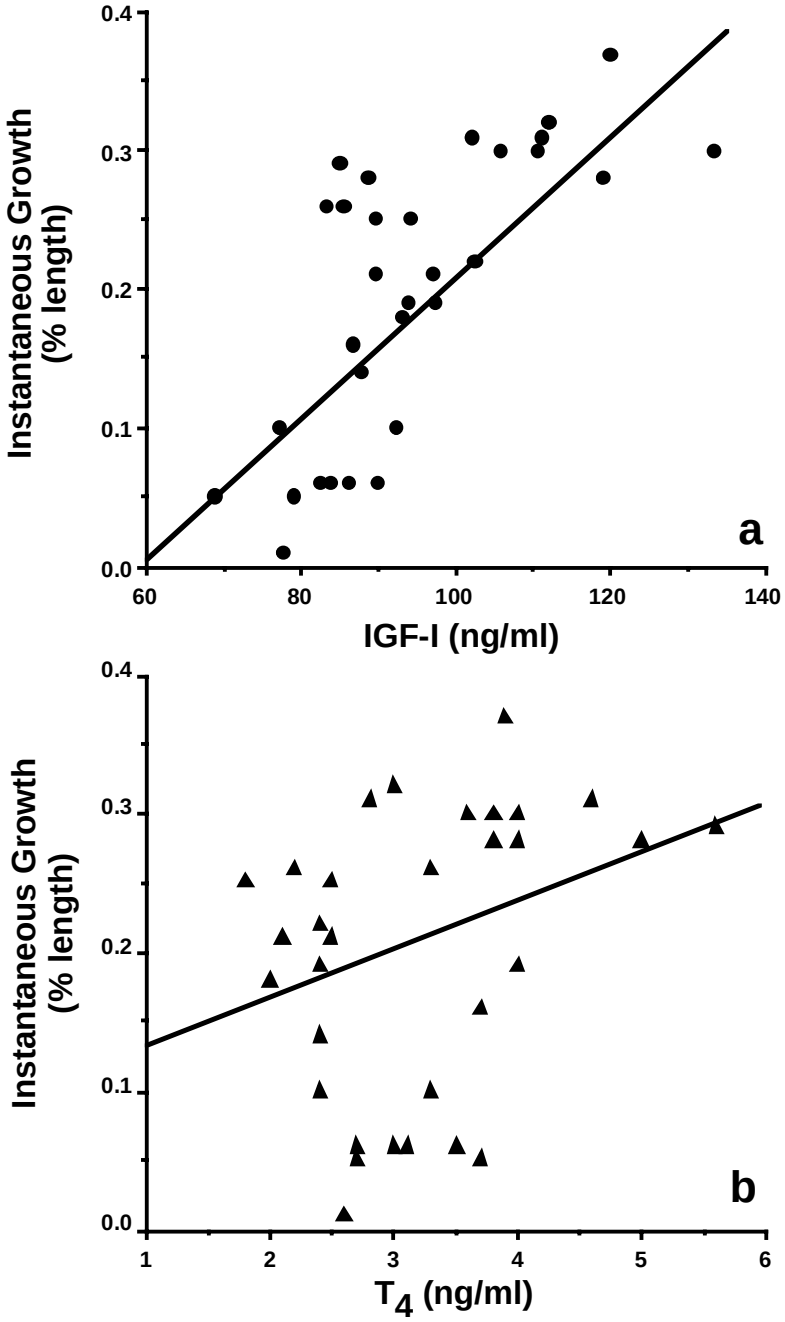

Fig. 9. Relationship between (a) instantaneous growth (% length) and plasma insulin-like growth factor-I (IGF-I) ($P < 0.001$, $R^2 = 0.50$) or (b) plasma thyroxine (T4) ($P = 0.06$, $R^2 = 0.12$). Each point represents the mean plasma hormone level found in a tank for a given growth interval (Jan-Feb, Feb-Mar, Mar-Apr, Apr-May), growth data generated from length data shown in Fig. 2.

stanzas, IGF-I levels measured at the beginning or the end of a stanza showed little relation to growth rate. Silverstein et al. (submitted) found elevated plasma IGF-I levels in chinook salmon fed at a higher relative ration; however, they found little relation between plasma IGF-I levels and growth rate. These data were collected from April through September, in contrast to our data collected from January through May. Removal of January data from our analysis resulted in a weaker relation between IGF-I and growth (low IGF-I values and low growth rates were found in January and served to drive the relation). In addition, regression analysis of IGF-I vs. growth were conducted in a different manner: average IGF-I values from a growth interval (this study) and IGF-I values from the end of a growth interval (Silverstein et al., submitted). One might not expect agreement over the relation of plasma IGF-I and growth between studies conducted over different seasons and in actuality analyzing different relations (average IGF-I levels over a growth period vs. IGF-I levels at the end of a growth period). Based on other studies (Pérez-Sánchez et al., 1994, 1995) one would expect an overall broad correlation between IGF-I and growth rate; however, this relation may disappear when it is examined on a smaller scale.

Our data do not show a clear pattern of IGF-I change in relation to physiological parameters of smoltification. A significant increase in IGF-I was found between January and February in all treatment groups. The LW and SW fish showed an additional increase in IGF-I in mid-March. Plasma IGF-I levels in SC and LC treatment fish stayed at a relatively constant level from February through May. Based on other measured physiological parameters, and in comparison to the LW and SW treatment groups, the LC and SC groups showed typical smolt development. The relatively few reports of IGF-I levels in smolting salmon (Lindahl et al., 1985; Duguay et al., 1994; Moriyama et al., 1994) suggest that there is a smoltification-associated rise in IGF-I. However, Moriyama et al. (1997) found no change from summer to fall in plasma IGF-I levels of fall smolting masu salmon (*O. masou*). None of these reports measured plasma IGF-I in chinook salmon. Thus, we are unable to ascertain the "typical" pattern of IGF-I change in plasma during smoltification of chinook salmon specifically, and it is yet unclear whether increases in plasma IGF-I are common during smoltification in any species.

Endocrine control of the parr-smolt transformation involves the GH-IGF-I axis, thyroid hormones, cortisol, and insulin, among others (Dickhoff, 1993). Administration of various hormones to stimulate smoltification suggests that GH is most effective (Miwa and Inui, 1985; Boeuf et al., 1994). Furthermore, it is well established that circulating levels of GH increase during smoltification (Sweeting et al., 1985; Young et al., 1989; Prunet et al., 1989; Boeuf et al., 1989). Stimulation of smoltification by increasing photoperiod is most likely mediated by GH (Komourdjian et al., 1976b; Björnsson et al., 1989; McCormick et al., 1995).

Many of the actions of GH are mediated by IGF-I produced in peripheral tissues. In salmon, the majority of circulating IGF-I comes from the liver (Duan et al., 1994). IGF-I is also produced in most other salmon tissues (Duguay et al., 1994) where they apparently have autocrine/paracrine effects and, for the most part, do not seem to be responsive to GH. In addition to the liver, other tissues that produce IGF-I in response to GH may include gill and kidney (Sakamoto and Hirano, 1993). The increase in hypo-osmoregulatory ability of salmon during smoltification appears to be mediated in part by

GH, since GH treatment results in increased hypo-osmoregulation upon transfer to seawater (Komourdjian et al., 1976b; Clarke et al., 1977; Miwa and Inui, 1985; Bolton et al., 1987; Collie et al., 1989; McCormick et al., 1991; Boeuf et al., 1994). Some of the osmoregulatory actions of growth hormone are mediated by IGF-I (Madsen and Bern, 1993; Sakamoto et al., 1993). Thus, a specific role of increases in IGF-I during smoltification may be to promote pre-adaptive salinity tolerance. The relative roles of circulating IGF-I versus IGF-I produced locally in gill or other osmoregulatory tissues remain to be established. In addition, the modulatory role of IGF-I binding proteins on IGF-I action promoting either growth in fishes or smoltification in salmonids is relatively unexplored (Kelley et al., 1992; Siharath et al., 1996).

We might have expected differences in $Na^+ K^+$ ATPase activity between warm and cool water groups, based on differences in growth and IGF-I in these same groups, as GH has been shown to stimulate somatic growth, plasma IGF-I, and $Na^+ K^+$ ATPase activity (Komourdjian et al., 1976a; Clarke et al., 1977; Sakamoto et al., 1993; Moriyama, 1995). All treatment groups showed a seasonal increase in $Na^+ K^+$ ATPase activity indicative of smoltification. The lack of difference in activity between groups suggests that either GH levels in all groups were high enough to maximally stimulate $Na^+ K^+$ ATPase activity or differences in plasma GH in our treatments were not different enough to produce differences in activity. Or, perhaps more realistically, that there isn't a simple relation between GH, IGF-I, growth, and $Na^+ K^+$ ATPase activity. An additional consideration of our results is that the cool water treatments showed a relatively high rate of growth as compared to other studies which have found differences in $Na^+ K^+$ ATPase activity as related to growth (Dickhoff et al., 1995). This suggests that a more stringent environmental manipulation may produce more discrete differences between these experimental variables. Several studies have manipulated photoperiod and found relations between increased growth and/or GH and $Na^+ K^+$ ATPase activity (Solbakken et al., 1994; Björnsson et al., 1995; McCormick et al., 1995).

The GH - IGF-I endocrine axis offers an intellectually appealing system for supplying developmental cues. Plasma GH levels change seasonally in response to photoperiod and temperature (Björnsson et al., 1995; McCormick et al., 1995). In addition, plasma IGF-I levels are subject to regulation by GH and are directly related to nutritional factors (Duan and Plisetskaya, 1993). Thus the GH - IGF-I axis may provide an integrated signal with regard to season, temperature, and food supply. Conditions found in the spring (increasing photoperiod, temperature and food supply) could up-regulate the GH-IGF-I system and in an organismal sense - suggest that environmental conditions are positive and favor development. Alternatively, decreasing photoperiod, temperature and scarce food resources would down-regulate this axis and signal winter conditions, retarding development.

A relationship between growth rate and animal development has been recognized in insects (Beck, 1971; Bradshaw and Johnson, 1995), amphibians (Wilbur and Collins, 1973; Alford and Harris, 1988), and fish (Policansky, 1983; Stearns, 1983). No published studies of smoltification have directly examined the influence of growth rate independent of body size, although several have suggested growth rate may play an important role (Wagner et al., 1969; Clarke et al., 1978; Thorpe, 1977; Metcalfe et al., 1988; Okland et al., 1993). An increasing growth rate during smoltification fits with the

pattern found in wild salmon, based on our studies of juvenile spring chinook salmon (Beckman, Larsen, and Dickhoff unpublished). Together these results suggest continued investigation on the role of the GH/IGF-I axis in regulating smoltification. Future studies will focus on creating more dramatic differences in environment which will presumably have greater effects on growth and smoltification.

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CHAPTER 3

"REARING A WILD – LIKE SMOLT"

Publication Title A: The Effect of Low Temperature and Fasting During the Winter on Growth and Smoltification of Coho Salmon.

Publication Title B. The Effect of Low Temperature and Fasting During the Winter on the Metabolic and Endocrine Physiology of Coho Salmon.

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A: The Effect of Low Temperature and Fasting During the Winter on Growth and Smoltification of Coho Salmon.

SUMMARY

This study examined the effect of winter feeding and fasting under both warm (10 ˚C) and cold (2.5 ˚C) water temperature, on smoltification of juvenile coho salmon, *Oncorhynchus kisutch*. Treatments were as follows: Warm-Fed, Warm-Not Fed, Cold-Fed, and Cold-Not Fed during the winter (January - February) prior to smoltification (March-May). All groups were fed and maintained at 10˚C during the smoltification period. The following parameters were measured: fork length, weight, condition factor, smolt-associated appearance, whole body lipid levels, and gill Na^+/K^+-ATPase activity. Warm-Fed fish grew continuously throughout the winter and were larger than fish from the other treatments. Fish from the other groups showed little or no growth during January and February. While condition factor decreased significantly in the winter-fasted groups under both warm and cold temperatures, winter whole body lipid levels and smoltification-associated gill Na^+/K^+-ATPase activities were not different between groups. These data suggest that winter fasting, even under relatively warm winter water temperatures, may not impair the condition or smoltification of hatchery-cultured coho salmon.

INTRODUCTION

The environmental rearing conditions of most hatchery fish differ substantially with regard to temperature profiles and nutrition from the streams and rivers in which the animals evolved. In some hatchery environments, seasonal temperature fluctuations may be minimal or nonexistent. Furthermore, fish are often fed relatively high rations throughout the year to maximize growth, especially under situations where water temperatures remain high. These rearing conditions may have a profound impact on the morphology and physiology of these animals.

To date, only a few studies have attempted to characterize the physiology of naturally reared salmon (Atlantic salmon *Salmo salar*-Youngson and Simpson 1984; McCormick and Bjornsson 1994; coho salmon *Oncorhynchus kisutch* -Rodgers et al. 1987; Ewing and Rodgers 1998). Recently, we conducted a relatively comprehensive examination of the physiology of naturally reared spring chinook salmon *O. tshawytscha* in the Yakima River of Washington State (Beckman et al., 2000). An obvious, yet significant, observation from that study was the very dynamic temperature conditions experienced by the fish during juvenile development. The water temperature reached 17-18 ˚C in July and August and steadily declined through the fall, reaching seasonal lows near 0 ˚C from December through February. In general, the amount of food in the stomach reflected this trend in temperature. High amounts of food were observed in the stomachs of fish in the spring, summer and early fall, whereas stomach fullness declined to near empty in the late fall and winter.

Based on these observations, the current investigation was designed to examine the effect of rearing fish in the laboratory under more variable winter environmental conditions on growth and smoltification (parr-smolt transformation) of coho salmon. Coho salmon typically undergo the parr-smolt transformation after over-wintering 1 year in fresh water, then migrate to the ocean in late spring (Sandercock 1991). Thus, we examined the effect of winter feeding and fasting under both warm (10 ˚C) and cold (2.5 ˚C) winter temperatures (January-February) on morphology, whole body lipid levels and gill Na^+/K^+-ATPase activity of coho salmon during the parr-smolt transformation (March-May). Our objective was to better understand how environmental manipulations affect smoltification of captively reared salmonids. Furthermore, it is generally believed that naturally reared salmonids display superior condition and survival compared with hatchery fish (see Wood et al 1957; Lindsay et al. 1989; Fast et al. 1991). Thus, our ultimate goal is to improve rearing protocols of captively reared fish such that their morphology and physiology might better approximate that of their naturally reared counterparts.

METHODS

Animals and rearing conditions

In November 1995, approximately 4,000 coho salmon eggs were obtained from the broodstock that returns yearly to the University of Washington, School of Fisheries hatchery, Seattle, Washington, and were reared at the Northwest Fisheries Science Center, Seattle, Washington. The fish were held in 1.3m diameter cylindrical fiberglass

tanks in a closed recirculation system with biofiltration, ozonation and ultraviolet sterilization. Fish density in tanks in November was 11.3 kg/m^3 (Density Index = 0.16; calculated according to Piper et al. 1982). Fish density at the end of May was 17.6 kg/m^3 (Density Index = 0.18). Water temperature was constant at 10 ˚C throughout the first year of rearing. Fish were fed Biodiet Grower Pellets (Bioproducts, Warrenton, Oregon) at the manufacturer's recommended rate of 1.5% body weight per day (bw/d) throughout the study unless otherwise noted (see below) using automatic belt feeders (Babington Enterprises, Inc., Hagerman, Idaho[1]). The fish were maintained under timer-controlled incandescent lights adjusted weekly to match the photoperiod of Seattle. Mortality throughout the entire study was less than 1%.

The experimental design is depicted in Figure 1 (A and B). In late November 1996 1,600 fish with an average body weight of 21.5g ± 0.3 (mean±SEM n=60/treatment)) were randomly selected from the original pool and assigned to one of four treatments (400 fish/treatment) in a two-by-two factorial design as follows: warm temperature (10 ˚C) and fed (1.5% bw/day) in January and February (Warm-Fed), warm temperature (10 ˚C) and fasted in January and February (Warm-Not Fed), cold temperature (2.5 ˚C) and fed (0.7% bw/day) in January and February (Cold-Fed), and cold temperature (2.5 ˚C) and fasted in January and February (Cold-Not Fed). For the low temperature treatments, fish were reared at 2.5 ˚C by passing the water through a counter-current glycol heat exchanger (APV Heat Exchangers, Tonawanda, New York).

Starting on January 6, 1997, the water temperature for the Cold-Fed and Cold-Not Fed treatments was gradually decreased over approximately 3 days to 2.5 ˚C. This temperature was maintained until March 3, 1997, at which time the temperature was gradually raised from 2.5 ˚C to 10 ˚C over a 3-day period. The Cold-Fed group was fed at the manufacturer's recommended level of 0.7% bw/d for the temperature of 2.5 ˚C until March 3. The Cold-Not Fed group was fasted for the same period. The Warm-Fed treatment continued to be fed at 1.5% bw/d at 10 ˚C throughout the study while the Warm-Not Fed treatment was fasted until March 3. After March 3, the Cold-Fed, Cold-Not Fed, and Warm-Not Fed treatments were all returned to the ration of 1.5% bw/d until the end of the experiment in June.

Sample collection and analysis

Fork length (mm) and body weight (g) were measured from 60 randomly selected fish from each treatment approximately monthly for adjusting ration and calculation of condition factor (CF). CF was calculated as $CF = w/l^3 \times 100$, where w = weight in g and l = length in cm. Approximately every 2 weeks, 12 fish from each treatment were individually anesthetized in a buffered solution of 0.05% tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, Washington) and visually assessed for stage of smolt development (1 = parr, 2 = transitional, 3 = smolt; modified from Gorbman et al., 1982). Gill tissue was sampled from mid-March to mid-May. Filaments from three gill arches were placed in a solution of sucrose, EDTA, and imidazole according to methods described by Zaugg (1982) and then frozen on dry ice and stored at -80 ˚C. Gill Na^+/K^+-ATPase activities were measured using the method of Schrock et al. (1994). Carcasses (excluding a portion of the plasma and the liver

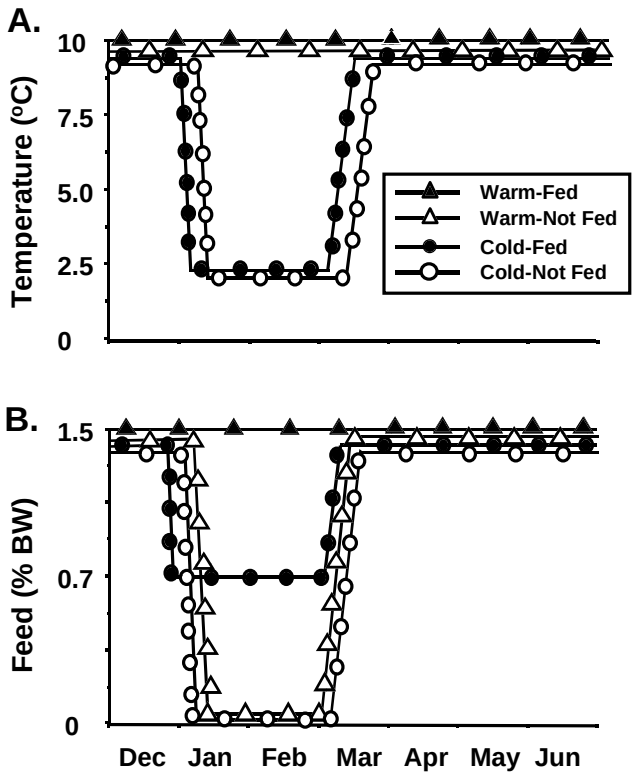

Figure 1 (A and B) Experimental temperature (A) and feeding (B) regime used to raise four treatment groups of coho salmon from December 1996 to June 1997. Treatments included: Warm-Fed (10 ˚C fed 1.5% bw/d), Warm-Not Fed (10 ˚C and fasted), Cold-Fed (2.5 ˚C fed 0.7% bw/d), and Cold-Not Fed (2.5 ˚C and fasted) from January 6, 1996, to March 3, 1997. Throughout the rest of the experiment all fish were reared at 10 ˚C and fed 1.5% bw/d.

which were collected for a companion study) for whole body lipid analysis were collected approximately monthly from early December to early May with more frequent sampling just after the temperature and ration changes were carried out in early January and early March. Whole body lipid levels were determined gravimetrically by the method of AOAC (1975) with lipid extracted using methylene chloride.

Statistical Analysis

Data were examined using a three-way analysis of variance (ANOVA) with date, temperature regime, and feeding regime being the effects modeled. If significant effects were found, differences between individual means (either between treatments for a given date or dates for a given treatment) were examined using one-way ANOVA followed by Fisher's protected least significant differences test (Dowdy and Weardon 1991). Significant differences among dates for a given treatment are indicated in the text.

Different letters on the figures indicate significant differences between treatments at a given date. All statistical analyses were conducted using Statview 512+ (Brain Power, Inc., Cupertino, California). Statistical significance was set at a level of $\alpha = 0.05$.

RESULTS

Growth and Morphology

Initial lengths (mean 113±0.6 mm, mean±SE) and weights (mean 22±0.3 g) of all four groups of fish were similar (Fig. 2 and 3). The Warm-Fed fish grew steadily in length and weight throughout the experiment to a maximum of 154±1.6 mm and 43±1.4 g respectively in late May. In contrast the Warm-Not Fed, Cold-Fed, and Cold-Not Fed fish did not grow in length or weight during the treatment period. After these three groups were returned to 10 ºC and a feed rate of 1.5% bw/d, all treatments showed significant increases in length and weight from early April to late May. The Warm-Fed fish were significantly longer and heavier than fish from the other three treatments starting in late February and remained so throughout the rest of the experiment. It is noteworthy that there were significant differences in both length and weight between the Warm-Not Fed, Cold-Fed, and Cold-Not Fed groups at various dates indicated by different letters at a given date throughout the experiment. However, these transient differences were relatively small compared with the difference between any of these groups and that of the Warm-Fed fish.

In mid-December, prior to initiation of the treatments, CF values of fish from the four treatments were not significantly different (1.31±0.01) (Fig. 4). Following the start of treatments, CF declined significantly in the Warm-Not Fed, Cold-Fed, and Cold-Not Fed groups through February while the CF of the Warm-Fed fish remained high and unchanging. The two unfed treatments showed the most pronounced decreases in CF while the Cold-Fed group was intermediate. In mid-March, when all groups were returned to 10 ˚C and 1.5% bw/d ration, the CF of the Warm-Not Fed group increased significantly to a level similar to that of the Warm-Fed treatment. The CF of the Cold-Fed and Cold-Not Fed groups increased significantly as well; however, the levels were not as high as that of the warm water groups. Finally, from late-March to mid-April the CF of all treatment groups decreased significantly and then remained unchanged through the remainder of the experiment. From late-March to mid-May there were significant differences in CF between some of the treatments with the Warm-Fed fish having the highest levels and the Cold-Not Fed fish the lowest; however, the pattern and the levels were relatively similar among all treatment groups. On the final sampling date in late-May there was no significant difference between any of the treatments.

There was a significant change in smoltification-associated appearance over time in all groups (Fig. 5). From early-December to late-February most fish in all treatments were classified as parr. From March to mid-April a large proportion of the fish was

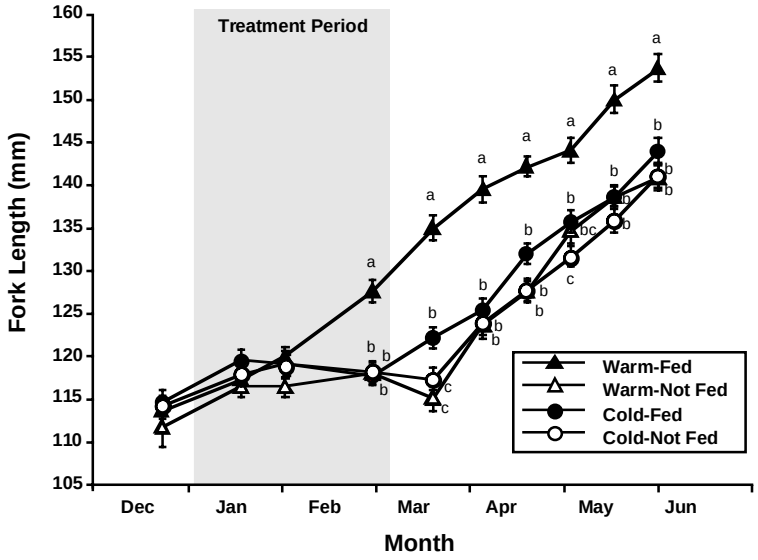

Figure 2. Fork length (mm) of juvenile coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=60 fish/treatment/date).

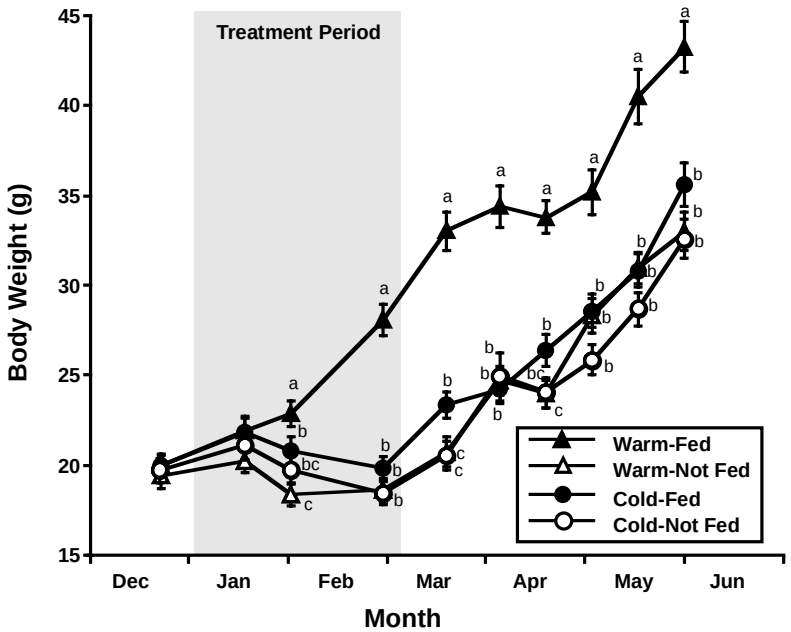

Figure 3. Body weight of juvenile coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=60 fish/treatment/date).

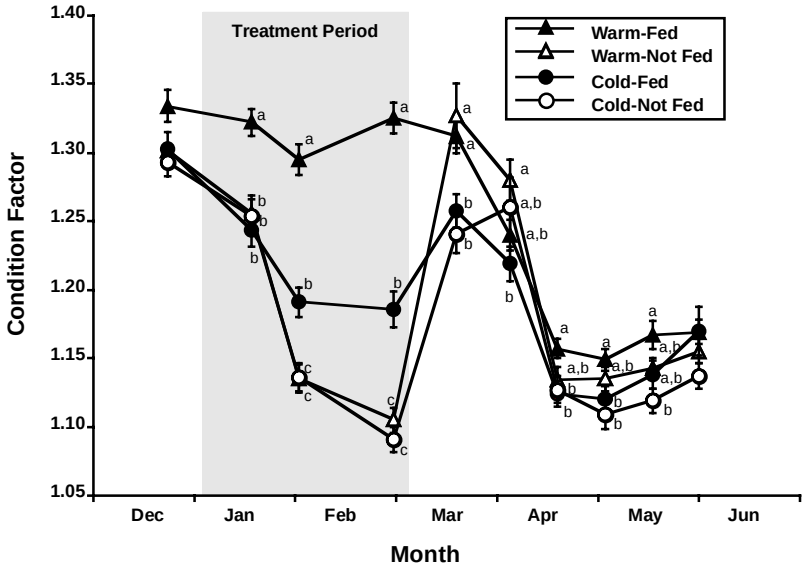

Figure 4. Condition factor of coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=60 fish/treatment/date).

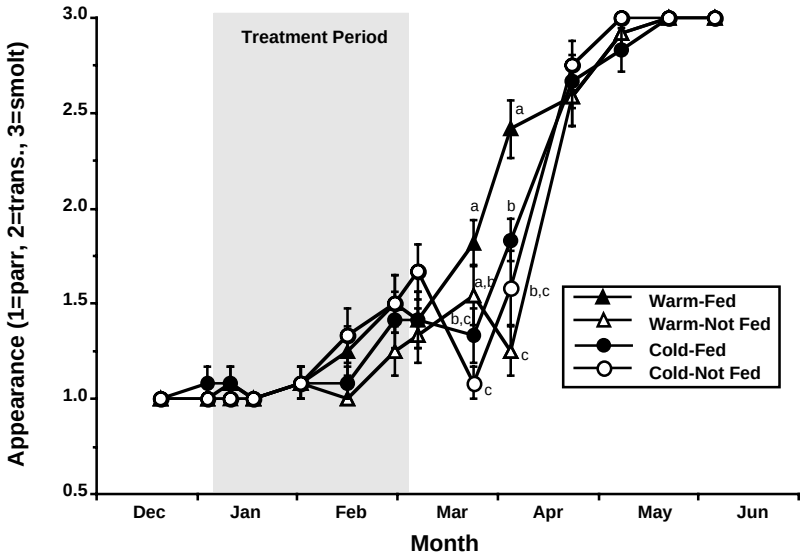

Figure 5. Appearance of coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. Fish were visually assessed for stage of smolt development based on the following scale: 1 = parr, 2 = transitional, 3 = smolt. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/date).

classified as transitional. There were significantly more transitional fish in the Warm-Fed group during this time as well. From late April to mid-May, most fish were classified as smolts. Finally, in late-May all fish in all treatments were classified as smolts.

Physiology

Whole body lipid levels changed very little throughout the experiment and there was little difference overall between treatments (Fig. 6). While there were significant differences among some dates within treatments, no distinct seasonal pattern was apparent in any of the treatments. At the beginning of the experiment lipid levels were quite high (approximately 11% wet body weight) in all fish. The levels remained relatively high in all groups throughout the experiment declining to only 7-9% in early April. Significant differences among treatments were found only between early March and early April with relatively higher levels seen in the Warm-Fed fish and relatively lower levels in the
Warm-Not Fed fish. The two groups maintained on cold water had intermediate lipid levels during the spring. The Warm-Not Fed group showed a significant decrease in lipid, to approximately 7% in early April; however, at the final sampling date in early May there was no significant difference among any of the treatments.

Gill Na^+/K^+-ATPase levels were measured from mid-March to mid-May (Fig. 7). ATPase activity varied significantly with season in all treatments from lows of 2.0-3.0 μmole PO_4 x mg pro^{-1} x hr^{-1} to peak levels of approximately 5.0-8.0 μmole PO_4 x mg pro^{-1} x hr^{-1} in mid-April. Overall, there were no significant differences in gill Na^+/K^+-ATPase activity among treatments.

DISCUSSION

Salmonid fishes have evolved in environments characterized by large seasonal fluctuations in water temperature and food availability. Coho salmon can withstand a temperature range of 0.5-25 ^oC with a much narrower optimum rearing temperature ranging from 10-14 ^oC (Piper et al. 1982). Our previous investigation of naturally reared spring chinook salmon found increased food consumption in the summer and low food consumption in the winter (Beckman et al., 2000). The objective of this study was to better understand how manipulations in winter temperature and feeding regime affect growth and the parr-smolt transformation of salmonids. We accomplished this goal using a factorial experimental design with what we perceived to be relatively extreme alterations of the animals' rearing environment during the winter prior to smoltification. We did not aim to mimic exactly the gradual seasonal changes the fish typically encounter, but rather to induce relatively extreme alterations in feeding and/or temperature regime in an effort to accentuate physiological responses (Fig. 1 A and B). These alterations, however, were conducted in the winter prior to smoltification, when day length is short, to approximate the time period in which such changes naturally occur.

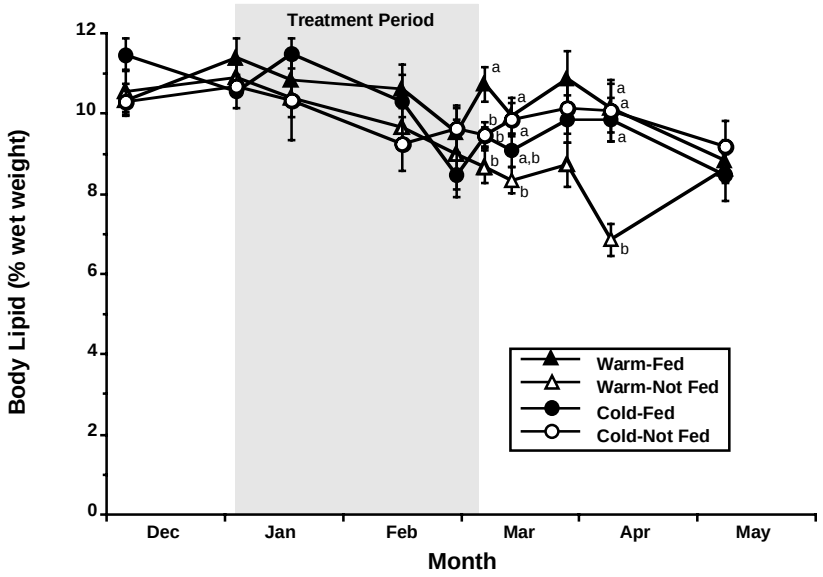

Figure 6. Whole body lipid levels (% wet weight) of coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. Samples were collected from early December to early May. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/date).

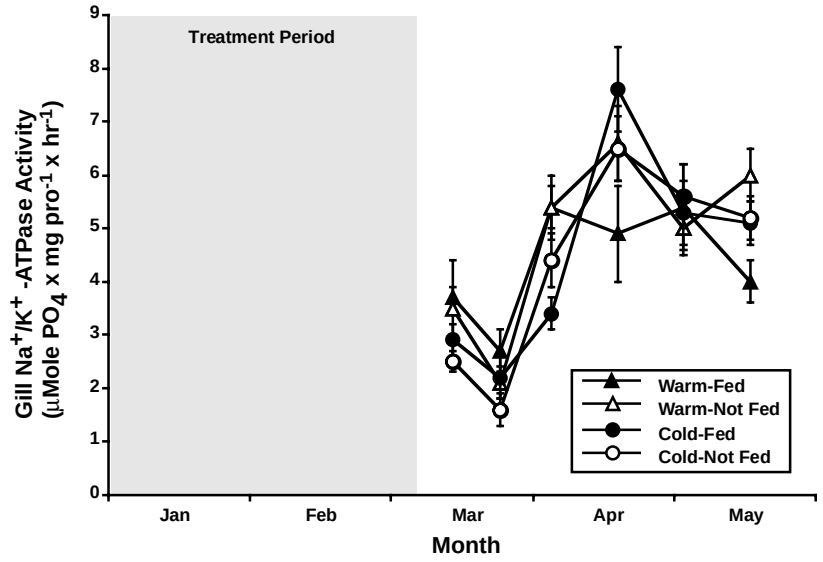

Figure 7. Gill Na^+/K^+ ATPase activity of coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. Gill samples were only analyzed in samples from early March to mid-May. The shaded treatment period indicates when temperature and feed manipulations were conducted (n=12 fish/treatment/date).

Furthermore, the temperatures were chosen to fall well within the normal physiological range of the animal and involved fasting as opposed to starvation, the latter being more physiologically detrimental involving breakdown in protein stores after lipid reserves have been exhausted. Akiyama and Nose (1980) showed that mortality due to starvation did not occur in juvenile chum salmon *O. keta* until whole body lipid levels approached less than approximately 2%. Lipid levels in naturally-rearing Yakima River spring chinook approached 2% in winter. The lowest lipid levels observed in this study were never below 7%. Thus, our diet treatment could not be considered starvation based on sustained lipid energy reserves and probably did not induce physiological damage.

One cannot completely rule out the contribution that tank effects may have had on the different treatment groups in this investigation. However, similar studies conducted in our laboratory using replicated treatment groups have not typically shown tank effects (for example see Beckman et. al.1998; Silverstein et. al.1998). In these and other studies, treatment and seasonal differences have a much greater effect than differences between replicate tanks. Furthermore, in this study the temperature and feed effects on growth and CF adhere to reasonable expectations, thus lending further support that the treatment effects are real and not solely attributable to tank differences.

Growth and Morphology

The Warm-Fed fish grew steadily throughout the experiment. By the end of February and continuing until the end of the experiment, these fish were both longer (Fig. 2) and heavier (Fig. 3) than all other groups. The other three treatments (Warm-Not Fed, Cold-Fed, and Cold-Not Fed) were all similar in both length and weight throughout the entire experiment. The aim of the Warm-Fed treatment group was to produce fish that were similar in size and growth profile to fish from a production hatchery that used a relatively constant temperature ground-water source. In this regard we were quite successful. However, it is worth noting that all of our treatment groups were relatively large with a initial mean weight of approximately 20 g and a initial mean length of approximately 110 mm in December. By the end of the experiment in June the Warm-Fed fish weighed 43±1.4 g at 153±1.6 mm in fork length while the other three groups weighed approximately 30-35 g at a length of 140-145 mm.

The CF of hatchery-reared populations of salmonids typically decreases from relatively high levels in the winter to low levels during smoltification in the spring as the animals become more streamlined (Hoar 1988). Beckman et al. (1999) showed that CF decreased from fall to winter in fish reared under ambient, but not constant ground water conditions. However, in that study, the ambient temperature fish showed little rebound in CF in the spring before declining during out-migration. In contrast, naturally rearing spring chinook salmon show a more complex pattern of change in CF (Beckman et al. 2000). In that study, CF was very high in late summer, declined to very low values in January-February before rebounding in early spring, and finally decreased once again during migration. A similar dynamic change in CF was observed in the present investigation most notably in the two fasted groups (Warm-Not Fed and Cold-Not Fed) and to a lesser degree in the Cold-Fed fish (Fig. 4). In contrast, the Warm-Fed fish showed little change in CF throughout the winter. This was expected as these fish were fed throughout the winter. However, regardless of the winter rearing conditions, all

groups underwent a similar decrease in CF during the final stages of smoltification. Thus, variation in rearing conditions, namely fasting, appeared to impart a CF profile most similar to that of wild salmonids.

Smolt Physiology

In general, the whole body lipid levels found in this study were quite high (7-12%) compared with levels observed in naturally reared Yakima River spring chinook (1-8%) (Beckman et al. 2000). However, they were very similar, in terms of both range and the minimal seasonal change, to levels observed in Yakima River spring chinook salmon reared in our experimental hatchery using standard commercial diets (Beckman et al. 1998). High lipid levels are common in hatchery-reared fish due to the high energy density of commercial diets and the typically high rations used (Shearer et al. 1997). The metabolic changes observed in this study are in general agreement with Leatherland and Nuti (1981) who found that in rainbow trout, *O. mykiss*, both hepatosomatic index and liver lipid content were significantly different between fed and fasted fish after 5, 10, 30, and 60 days. But, muscle lipid content was only different after 60 days and whole carcass lipid content decreased, but was not significantly different at any time. The livers were removed from the fish in the present investigation for liver glycogen analysis in a companion study. However, lipid reserves from the liver may have contributed minimally to the whole body lipid levels. In fact, in rainbow trout most lipid is preferentially deposited in perivisceral adipose tissue (Sargent et al. 1989). Jezierska et al. (1982) demonstrated that during a 48-d fast in rainbow trout, this mesenteric fat is mobilized from perivisceral depots before either liver or muscle fat. Finally, additional metabolic needs may have been met through breakdown of protein as well (see Navarro and Gutierrez 1995).

It was surprising to observe only a maximum decrease of approximately 30% in whole body lipid levels in association with both fasting and smoltification in this study. Nonetheless, the present data demonstrate that coho salmon, starting with high lipid stores, show little depletion of fat in response to fasting for two winter months, even at a relatively high winter temperature. Hilton (1982) demonstrated in rainbow trout that the pre-fasting diet may exert substantial influence on the metabolic events that take place during fasting. It remains to be determined whether this same conservation of lipid would be possible under natural rearing conditions with the added energetic demands of foraging, predator avoidance, smoltification, and migration. Also, during other times of the year, when the animals are evolved to undergo periods of significant growth, fasting may produce very different results and perhaps be detrimental to the animal.

High quality smolts are characterized by, among other factors, successful osmotic and ionic regulation in the marine environment (Hoar 1988). Gill Na^+/K^+-ATPase activity is commonly used as a benchmark physiological indicator of successful smoltification. Several studies have shown positive relations between ATPase activity and smolt-to-adult return (Ewing and Birks 1982; Zaugg 1989; Zaugg and Mahnken 1991; Beckman et al. 1999) while others have shown an association between seasonally increasing ATPase and performance characters such as seawater tolerance (Saunders and Henderson 1978; Boeuf and Harache 1982) and migratory readiness (Hart et al. 1981; Zaugg 1981a,b, 1989; Muir et al. 1994).

In the present study, there were no significant differences in gill Na^+/K^+-ATPase profiles among treatments throughout the spring (Fig. 7). Furthermore, based on smolt-associated appearance (Fig. 5), all treatment groups appeared to undergo smoltification in a similar manner in the spring. These results were somewhat surprising in light of the relatively dramatic differences in winter rearing regime among treatments. However, Beckman et al. (1998) also showed similar ATPase activities in chinook salmon reared under different spring temperatures with significant differences in growth. Results from this investigation suggest that salmonids are able to withstand relatively dramatic manipulation in winter rearing conditions, including low temperature and fasting, for an extended period of time with little effect on subsequent ATPase activity.

Our findings have potential application to both conservation and production salmon hatcheries. A goal of conservation hatcheries is to minimize domestic selection by matching the wild fish phenotype, including seasonal variation in growth rates (NWPPC, 1999). We have shown that low winter temperatures and low feeding can be applied without measurable detrimental effects on coho salmon. A widespread practice in production hatcheries is to reduce feeding rate during the winter because the fish show an apparent reduced appetite or less aggressive feeding behavior. Reducing winter rations could be an effective and economical approach in salmon hatcheries. Our results provide some experimental support for reducing winter rations. However, it is important to emphasize that this study was conducted at the laboratory scale and additional controlled studies are needed in both the laboratory and on a production scale to fully evaluate the applicability of these techniques.

Conclusions

Naturally rearing salmonids inhabit an environment with seasonal fluctuations in both temperature and food availability. In some hatchery environments, fish may experience much less fluctuation in these factors. The Warm-Fed treatment represented a hatchery group reared on constant temperature water and high ration. By exposing some groups of fish to more dynamic rearing conditions, namely low temperature with or without food in the Cold-Fed and Cold-Not Fed groups, we wanted to determine if we could rear a smaller, but still physiologically competent smolt. Finally, the Warm-Not Fed treatment was included to complete the two-by-two factorial design and allowed us to examine the effect of ration restriction in a hatchery environment where temperature may not be manipulated. Whole body lipid levels and gill ATPase activity were not different among treatments despite differences in CF and size. Furthermore, based on appearance, all treatments underwent the typical silvering associated with smoltification at relatively similar rates. This does not necessarily mean there were not differences in the fitness of these groups since we did not measure performance such as downstream migration or adult return. The lack of significant differences among treatments in the spring suggests that the fish are capable of withstanding long periods without feed during the winter even under relatively warm temperatures with no apparent detrimental effect on their ability to undergo smoltification. However, these animals had very high lipid stores when they entered this study. Similar manipulations using leaner fish may produce different results. From a hatchery perspective, feeding fish in the winter under warm

temperatures only created larger fish, not necessarily physiologically superior smolts. Clearly, further investigation is necessary using fish with metabolic stores more typical of those found in naturally reared animals taking in to consideration species differences, and a wider range of temperatures and feeding regimes.

ACKNOWLEDGEMENTS

We thank Brad Gadberry for conducting the lipid analysis. We also thank Dr. Erika Plisestskaya of the School of Aquatic and Fisheries Science, University of Washington, Seattle, Washington for a thoughtful review of this work.

B. The Effect of Low Temperature and Fasting During the Winter on the Metabolic and Endocrine Physiology of Coho Salmon.

SUMMARY

The objective of this study was to examine the effect of winter feeding and fasting at both high (10ºC) and low (2.5ºC) temperatures on growth, metabolic stores, and endocrinology of coho salmon. Treatments were as follows: Warm-Fed, Warm-Not Fed, Cold-Fed, and Cold-Not Fed during the winter (January-February). The following parameters were measured: length, weight, whole body lipid, liver glycogen, hepatosomatic index (HSI), and plasma levels of insulin, insulin-like growth factor-I (IGF-I), and thyroxine (T4). Warm-Fed fish grew continuously throughout the experiment from 21.5±0.3 to 43.4±1.4 g and were larger than the other treatments. All other treatments grew from 21.5±0.3 to approximately 32 g, and showed depressed growth during January and February. During the winter liver glycogen, hepatosomatic index, plasma insulin, and IGF-I were highly influenced by manipulations in rearing conditions whereas whole body lipid and plasma T4 were less affected. Plasma insulin levels fluctuated dramatically (from 2 up to 7 ng/ml) in the two cold acclimated groups shortly after the change in temperature. In general, the plasma insulin levels of the Warm-Fed fish were the highest (8-9 ng/ml), the Warm-Not Fed fish the lowest (2-5 ng/ml) and the two cold acclimated groups more variable but intermediate. In contrast, plasma IGF-I levels showed a decline with temperature decrease (from 9 down to 5 ng/ml) and more gradual changes than insulin with the change in feeding. The highest plasma IGF-I levels were found in the Warm-Fed fish (10-15 ng/ml), the lowest in the Cold-Not Fed fish (4-5 ng/ml), and those of the Warm-Not Fed and Cold-Fed fish were intermediate. During the treatment period the T4 levels were relatively unaffected by manipulations in feeding and temperature compared with either insulin or IGF-I. These data suggest that the insulin, IGF-I, and thyroid axes are differentially regulated under changing seasonal and/or environmental conditions in yearling salmon.

INTRODUCTION

The endocrine control of physiological processes in salmonids has evolved within the context of a seasonally varying environment. Unfortunately, many studies of endocrine physiology in salmonids have been conducted under conditions of relatively constant water temperature and feeding rate. These rearing conditions may have a profound impact on the morphological and physiological profiles of the animals. Only by studying fishes in a varying environment may endocrine and physiological responses be fully elucidated and their adaptive significance understood.

The endocrine physiology of naturally rearing salmon has been examined in only a few studies (Atlantic salmon, *Salmo salar*-Youngson and Simpson, 1984; McCormick and Bjornsson, 1994; coho salmon, *Oncorhynchus kisutch*-Rodgers *et al.*, 1987; Ewing and Rodgers, 1998). Recently, Beckman *et al.* (2000) examined the physiology of naturally rearing spring chinook salmon, *O. tshawytscha*, in the Yakima River of Washington State. One of the significant, but perhaps under-appreciated, observations from that study was the very dynamic temperature conditions experienced by these fish during juvenile development. Water temperature ranged from less than 1ºC (December-February) to as high as 18ºC (July-August). Food consumption followed this trend; being high in the spring and summer and relatively lower during the winter. Associated with these significant fluctuations in temperature and food consumption were very dynamic changes in the seasonal profiles of many of the physiological parameters examined including: growth rate, condition factor (CF), whole body lipid, hepatosomatic index (HSI), plasma thyroxine (T4) and insulin-like growth factor-I (IGF-I), and gill Na^+/K^+-adenosine triphosphatase (Na^+/K^+-ATPase) activity. Taking these observations from the field into consideration, the current investigation was designed to examine the effect of rearing fish in the hatchery under more extreme winter environmental conditions on size and metabolic and endocrine physiology.

Coho salmon typically undergo the parr-smolt transformation after over-wintering one year in freshwater then migrate to the ocean in late spring (Sandercock, 1991). Thus, we examined the effect of feeding and fasting, under both high (10ºC) and low (2.5ºC) winter temperature on size, metabolic stores (liver glycogen, whole body lipid) and endocrinology (plasma insulin, IGF-I, and T4) of coho salmon both before and during the parr-smolt transformation. The length, weight, and whole body lipid levels from this study are also presented in a related study on smoltification along with smolt-associated appearance, condition factor and gill Na^+/K^+-ATPase activity by Larsen et al. (2001) (See Chapter 3 A above). The ultimate goal of this work is to improve our understanding of the endocrine physiology of salmonid fishes within the context of a varying environment.

MATERIALS AND METHODS

Animals and experimental design

In November 1995, approximately 4,000 coho salmon eggs were obtained from the broodstock that returns yearly to the University of Washington, School of Aquatic and Fishery Sciences hatchery (Seattle, WA) and reared at the Northwest Fisheries Science Center (Seattle, WA). The fish were held in 1.3m diameter cylindrical fiberglass

tanks in a closed recirculation system with biofiltration, ozonation and UV sterilization. Fish density in tanks in November was 11.3 kg/m^3 (Density Index = 0.16; calculated according to Piper *et al.,* 1982). Fish density at the end of May was 17.6 kg/m^3 (Density Index = 0.18). Water temperature was a constant 10ºC throughout the first year of rearing. Fish were fed Biodiet Grower Pellets (Bioproducts, Warrenton, OR) at the manufacturer's recommended rate of 1.5% body weight per day (bw/d) throughout the study unless otherwise noted (see below) using automatic belt feeders (Babington Enterprises, Inc., Hagerman, ID). Fish were maintained under timer-controlled incandescent lights adjusted weekly to match the photoperiod of Seattle. Mortality throughout the entire study was less than 1%.

The experimental design is depicted in Figure 1. In late November 1996 1,600 fish with an average body weight of 21.5g ± 0.3 (mean±SEM n=60/treatment) were randomly selected from the original pool and assigned to one of four treatments (400 fish/treatment) in a two-by-two factorial design as follows: warm temperature (10ºC) and fed (1.5% bw/day) in January and February (Warm-Fed or Control), warm temperature (10ºC) and fasted in January and February (Warm-Not Fed), cold temperature (2.5ºC) and fed (0.7% bw/day) in January and February (Cold-Fed), and cold temperature (2.5ºC) and fasted in January and February (Cold-Not Fed). For the low temperature treatments, fish were reared at 2.5ºC by passing the water through a counter-current glycol heat exchanger (APV Heat Exchangers, Tonawanda, NY).

Starting on January 6, 1997, the water temperature for the Cold-Fed and Cold-Not Fed treatments was gradually decreased over approximately 3 days to 2.5ºC. This temperature was maintained until March 3, 1997, at which time the temperature was gradually raised from 2.5ºC to 10ºC over a 3-day period. The Cold-Fed group was fed at the manufacturer's recommended level of 0.7% (bw/d) for the temperature of 2.5ºC until March 3. The Cold-Not Fed group was fasted for the same period. The Warm-Fed or Control treatment, continued to be fed at 1.5% (bw/d) at 10ºC throughout the study while the Warm-Not Fed treatment was fasted until March 3. After March 3, the Cold-Fed, Cold-Not Fed, and Warm-Not Fed treatments were all returned to the ration of 1.5% (bw/d) until the end of the experiment in June. Fish rearing and treatment protocols were with approval and in accordance with guidelines of the Animal Care Committee, University of Washington.

Sample collection

Fork length (mm) and body weight (g) were measured from 60 randomly selected fish from each treatment approximately monthly to accurately estimate mean size of fish and for adjusting ration. The frequency with which samples were collected varied depending on the tissue. Twelve fish were randomly selected from each treatment at the same time of day; 0900-1200-approximately 16 hrs after last feeding. Fish were individually anesthetized in a buffered solution of 0.05% tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, WA). Blood samples were collected approximately every two weeks from December to early June from severed caudal vessels into heparinized Natelson tubes (VWR Scientific), centrifuged for 3 minutes at 3000 g, and stored frozen at -80ºC until analyzed by radioimmunoassay. Livers for glycogen content and determination of HSI and carcasses for whole body lipid analysis

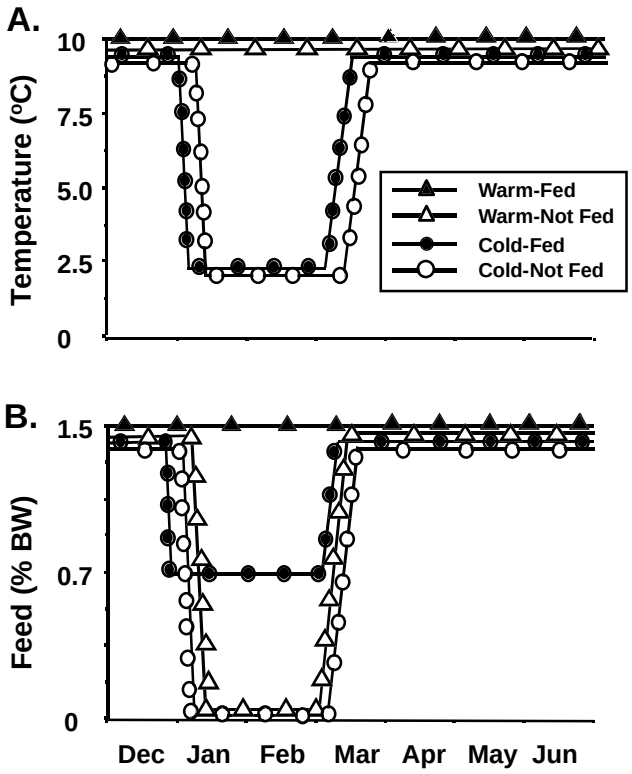

Figure 1 (A and B) Experimental temperature (A) and feeding (B) regime used to raise four treatment groups of coho salmon from December 1996 to June 1997. Treatments included: Warm-Fed (10 ˚C fed 1.5% bw/d), Warm-Not Fed (10 ˚C and fasted), Cold-Fed (2.5 ˚C fed 0.7% bw/d), and Cold-Not Fed (2.5 ˚C and fasted) from January 6, 1996, to March 3, 1997. Throughout the rest of the experiment all fish were reared at 10 ˚C and fed 1.5% bw/d.

were collected approximately monthly from early December to early May with more frequent sampling just after the temperature and ration changes were carried out in early January and early March. Livers were weighed, placed in HistoPrep tissue cassettes (Fisher Scientific, Pittsburgh, PA), frozen in liquid nitrogen, and stored at -80ºC until analyzed. The remaining fish carcasses were placed in individual plastic bags and frozen at -80ºC until analyzed for whole body lipid. HSI was calculated as: liver weight (g) * body weight^{-1} * 100. Smoltification associated appearance, condition factor, and gill Na^+/K^+-ATPase activity were also determined from these fish for a related study (Larsen *et al.*, 2001).

Sample analysis

Plasma insulin levels were measured in individual samples at each sampling date (n=12) using a homologous salmon radioimmunoassay according to the method of Plisetskaya *et al*. (1986). Due to the small amount of plasma available from individual

fish following measurement of plasma insulin levels, the plasma had to be pooled from two fish (n=6 pools from 12 fish per date) for measurement of plasma T4 and IGF-I. Plasma T4 concentrations were determined according to the method of Dickhoff *et al.* (1982). Total plasma IGF-I levels were measured following acid-ethanol extraction (Daughaday *et al.,* 1980) with recombinant salmon IGF-I and anti-Barramundi IGF-I serum obtained from Gro-Pep Inc. (Adelaide, Australia) according to Shimizu *et al.* (2000). Liver glycogen content was determined by the method described by Plisetskaya *et al.* (1994). Whole body lipid was determined by the method of AOAC (1975) with lipid extracted using methylene chloride.

Statistical Analysis

Data for each parameter were examined using a three-way analysis of variance (ANOVA) with date, temperature regime, and feeding regime being the effects modeled. If significant effects were found, differences between individual means (either between treatments for a given date or dates for a given treatment) were examined using one-way ANOVA followed by Fisher's protected least significant differences test (Dowdy and Weardon, 1991). Significant differences among dates for a given treatment are indicated in the text. Different letters on the figures indicate significant differences between treatments at a given date. All statistical analyses were conducted using Statview 512+ (Brain Power, Inc., Cupertino, CA). Statistical significance was set at a level of $\alpha = 0.05$.

RESULTS

Growth

Initial lengths (mean 112.6±0.6 SEM mm) and weights (mean 21.5±0.3 SEM g) of all four groups of fish were similar (Fig. 2 and 3). All treatment groups showed a significant effect of date on length and weight throughout the experiment. Warm-Fed fish grew steadily in length and weight throughout the experiment to a maximum of 153.8±1.6 mm and 43.4±1.4 g in late May. In contrast Warm-Not Fed, Cold Fed, and Cold-Not Fed fish did not grow in length or weight during the treatment period. After these three groups were returned to 10ºC and fed 1.5% (bw/d), fish steadily increased in length and weight from early April to late May. Warm-Fed fish were significantly longer and heavier than fish from the other three treatments starting in late February and remained so throughout the rest of the experiment. It is noteworthy that there were significant differences in both length and weight between the Warm-Not Fed, Cold-Fed, and Cold-Not Fed groups at various dates indicated by different letters at a given date throughout the experiment. However, these transient differences were relatively small compared with the difference between any of these groups and that of the Warm-Fed fish.

Metabolic Stores

Liver glycogen levels were not significantly different between treatments prior to the initiation of the temperature and feed manipulations (Fig. 4). There was a significant

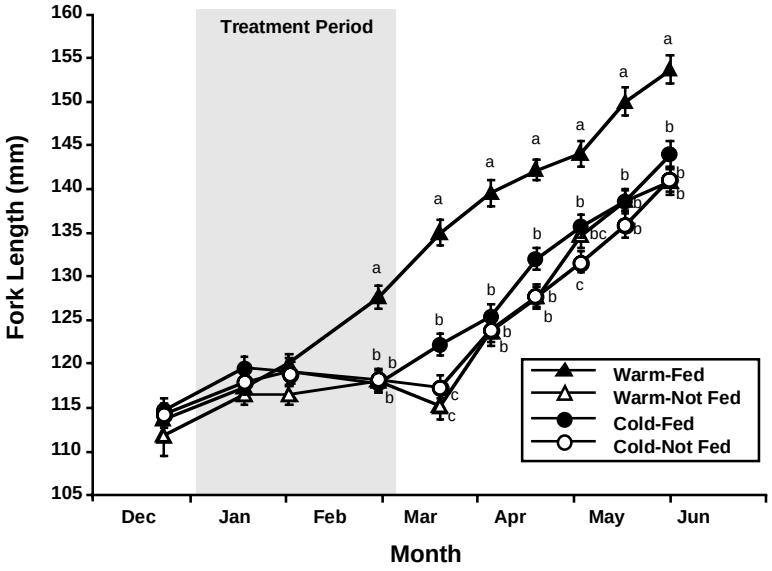

Figure 2. Fork length (mm) of juvenile coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=60 fish/treatment/date).

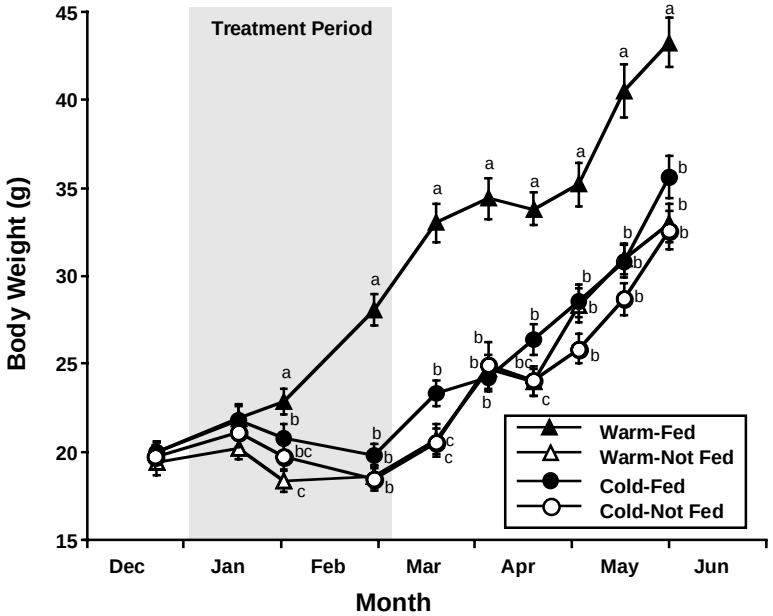

Figure 3. Body weight of juvenile coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=60 fish/treatment/date).

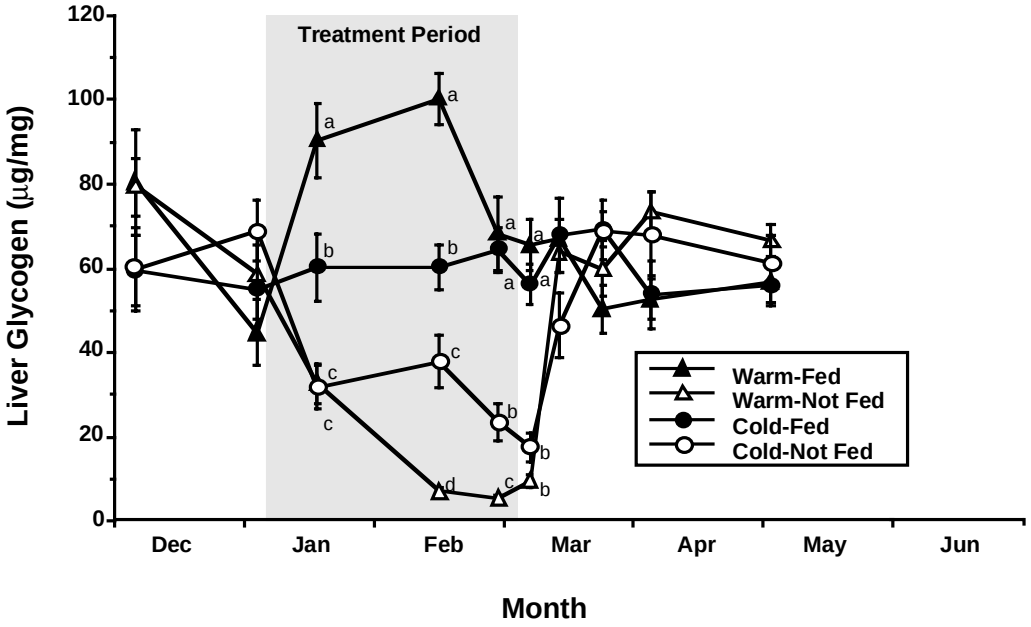

Figure 4. Liver glycogen levels of coho salmon raised under four temperature/feeding regimes. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/date).

effect of date on liver glycogen levels of the Warm-Fed, Warm-Not Fed, and Cold-Not Fed fish, but not the Cold-Fed fish. Within one week following the change in rearing regime, there were significant differences in liver glycogen between treatments. In the Warm-Fed fish liver glycogen increased significantly from 45 (μg/mg) in early January to approximately 100 (μg/mg) from mid-January to mid February despite the fact that they were reared under constant temperature (10ºC) and ration (1.5% bw/d). In late-February liver glycogen levels in the Warm-Fed fish declined to approximately 60 (μg/mg) and remained relatively constant for the rest of the experiment. In contrast, liver glycogen remained relatively unchanged at a level of approximately 55 (μg/mg) throughout the entire experiment in the Cold-Fed fish. Finally, liver glycogen levels in both the Warm-Not Fed and Cold-Not Fed treatments declined significantly during the treatment period and remained low throughout January and February. This decline was most dramatic in the Warm-Not Fed fish. However, in early-March, when all groups were returned to 10ºC and 1.5% (bw/d), the Warm-Not Fed and the Cold-Not Fed groups showed a dramatic increase in liver glycogen to levels comparable to the Warm-Fed and Cold-Fed fish. From mid-March to the end of the experiment in late May there was no significant difference in liver glycogen between any of the treatments.

The changes in hepatosomatic index (HSI) closely mirrored those of liver glycogen from the beginning of the experiment in December until the end of February (Fig. 5). There was a significant effect of date on HSI in all treatment groups. HSI

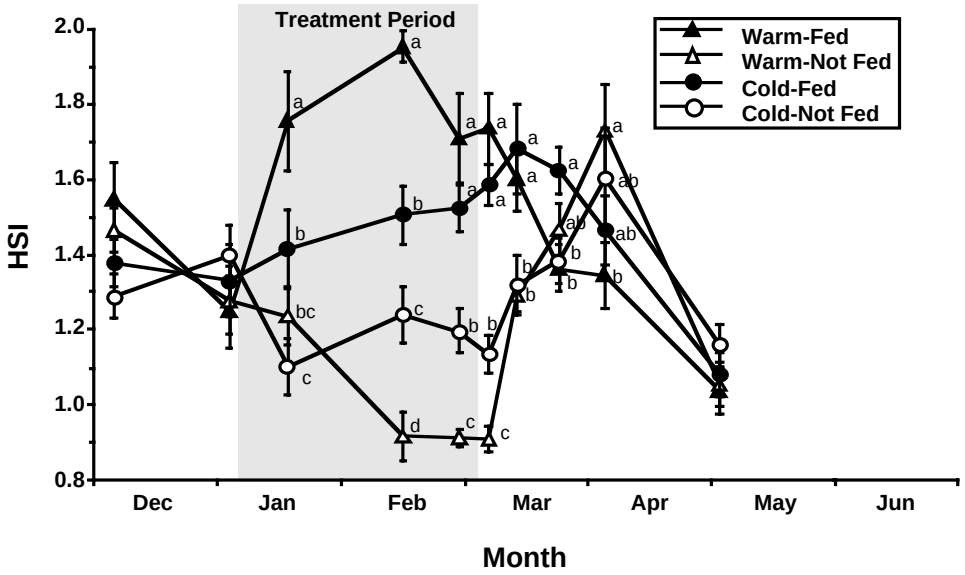

Figure 5. Hepatosomatic index (HIS) coho salmon raised under four temperature/feeding regimes. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/date).

increased significantly in the Warm-Fed fish during the winter before decreasing dramatically starting in mid-February. HSI showed a small, but significant increase in the Cold-Fed fish through early March. Finally, HSI decreased significantly in the Warm-Not Fed and Cold-Not Fed groups during the winter before increasing in early March when these two groups were refed. By late-March all treatments had similar HSI levels of approximately 1.4 before decreasing significantly by early-May to a value of approximately 1.1.

At the beginning of the experiment lipid levels were quite high (approximately 11% wet body weight) and there were no significant differences between the four groups (Fig 6.). There was a significant effect of date on whole body lipid levels of the Warm-Fed, Warm-Not Fed, and Cold-Not Fed fish, but not the Cold-Not Fed fish. While there were significant differences among some dates within treatments, the seasonal changes were minimal (1-2%) and no distinct seasonal pattern was apparent in any of the treatments. The levels remained relatively high in all groups throughout the experiment declining to only 7-9% in early April. Significant differences among treatments were found only between early March and early April with relatively higher levels seen in the Warm-Fed fish and relatively lower levels in the Warm-Not Fed fish. The two groups maintained on cold water had intermediate lipid levels during the spring. The Warm-Not Fed group showed the greatest decrease in lipid, to approximately 7% in early April; however, at the final sampling date in early-May there was no significant difference among any of the treatments.

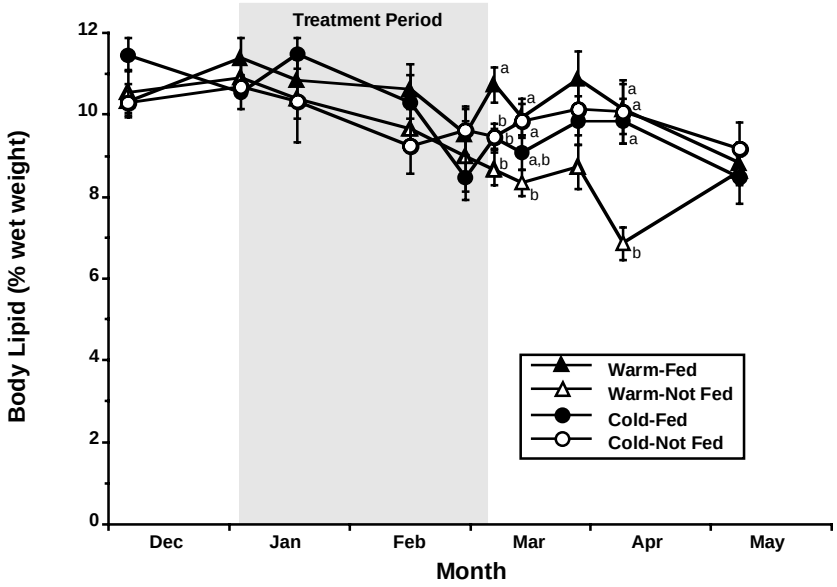

Figure 6. Whole body lipid levels (% wet weight) of coho salmon raised under four temperature/feeding regimes from December 1996 to June 1997. Samples were collected from early December to early May. The shaded treatment period indicates when temperature and feed manipulations were conducted. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/dat

Endocrine Physiology

Insulin levels were not significantly different among treatments (approximately 2.0-4.0 ng/ml) prior to the change in rearing conditions (Fig. 7). There was a significant effect of date on plasma insulin levels in all treatments. Within one week following the drop in temperature from 10ºC to 2.5ºC in early January, plasma insulin levels showed a dramatic transient increase to over 7.0 ng/ml in the two cold temperature groups. In contrast, during this same period they remained unchanged (Warm-Fed) or significantly decreased (Warm-Not Fed) in the warm temperature groups. In February, the Warm-Fed fish showed a steady increase in insulin to a peak of 6.7±1.8 ng/ml before declining in late March. During this same period, the insulin levels of the Warm-Not Fed fish continued to decline to levels below 1.0 ng/ml. In contrast, the insulin levels of the two cold temperature groups remained relatively higher than the Warm-Not Fed fish ranging between 2.0 to 5.0 ng/ml for all but one sampling date. The Cold-Fed fish had consistently higher insulin levels than the Cold-Not Fed fish. In early March, when all groups were returned to a temperature of 10ºC and a ration of 1.5% (bw/d), significant differences among treatments were also found. As fish were returned to feeding, insulin in the Warm-Not Fed fish increased steadily to 4.0 ng/ml, which was similar to the level of the Warm-Fed fish at this time. Insulin levels in the Cold-Fed fish showed a dramatic, transient increase to 8.8±2.1 ng/ml before declining to approximately 5.0 ng/ml in late March. Finally, the Cold-Not Fed fish showed an increase in plasma insulin from

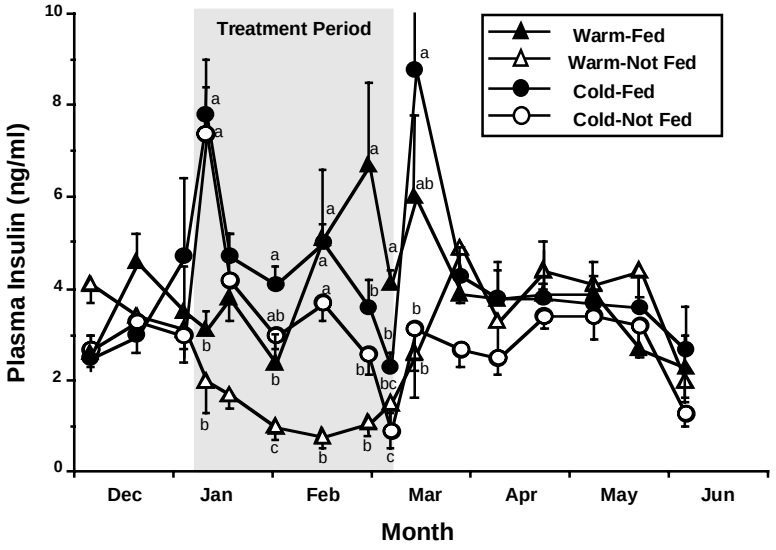

Figure 7. Plasma insulin levels of coho salmon raised under four temperature/feeding regimes. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=12 fish/treatment/date).

approximately 1.0 up to 3.0 ng/ml over this same period. During April and May plasma insulin levels were not significantly different among treatments and remained between approximately 1.0 and 4.0 ng/ml.

Plasma IGF-I levels varied significantly with date in all treatments (Fig. 8). In December, prior to the treatment manipulations, plasma IGF-I levels were not significantly different between treatments, ranging from approximately 7.0-9.0 ng/ml. However, shortly after the change in rearing conditions in January, IGF-I levels remained unchanged in the warm groups and decreased in the cold groups despite differences in feeding. By February IGF-I in the Warm-Not Fed group declined to levels similar to the cold groups. In contrast the Warm Fed fish showed a steady increase in IGF-I throughout the winter up to a maximum of 10-15 ng/ml in early March. After all treatments were returned to 10ºC and a ration of 1.5% (bw/d), IGF-I levels increased steadily throughout March, first in the Cold-Fed fish, then the Warm-Not Fed fish and finally, after some lag, in the Cold-Not Fed fish. In early to mid-April plasma IGF-I levels plateau in the Warm-Fed fish and peaked in all other treatments before plateauing or declining through May. During April and May there were no significant differences in plasma IGF-I levels between any of the treatments.

Plasma T4 levels varied significantly with date in all treatments, being lowest in January and February and peaking in April-May (Fig. 9). In December, prior to the treatment manipulations, plasma T4 levels were not significantly different among treatments, ranging from 4.0-5.0 ng/ml. Following the change in temperature and ration, T4 levels remained relatively low in all groups including the Warm-Fed fish. There was,

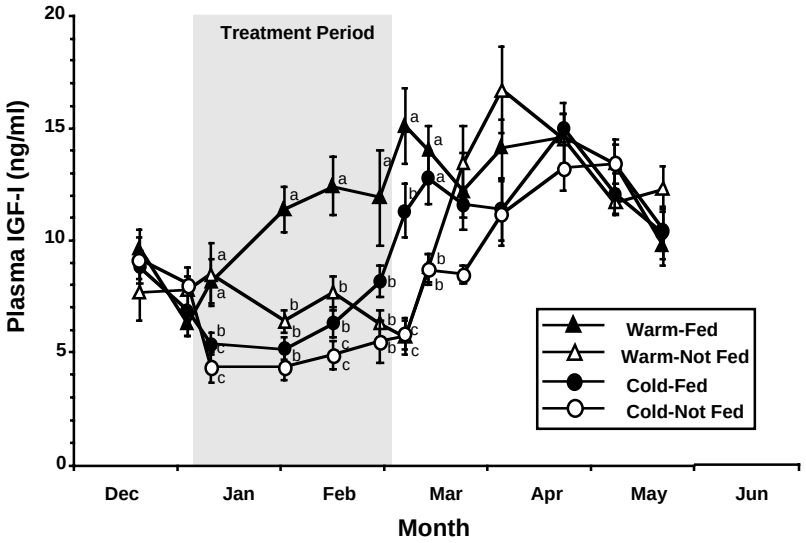

Figure 8. Plasma insulin-like growth factor -I (IGF-I) levels of coho salmon raised under four temperature/feeding regimes. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=6 pools of 2 fish/treatment/date).

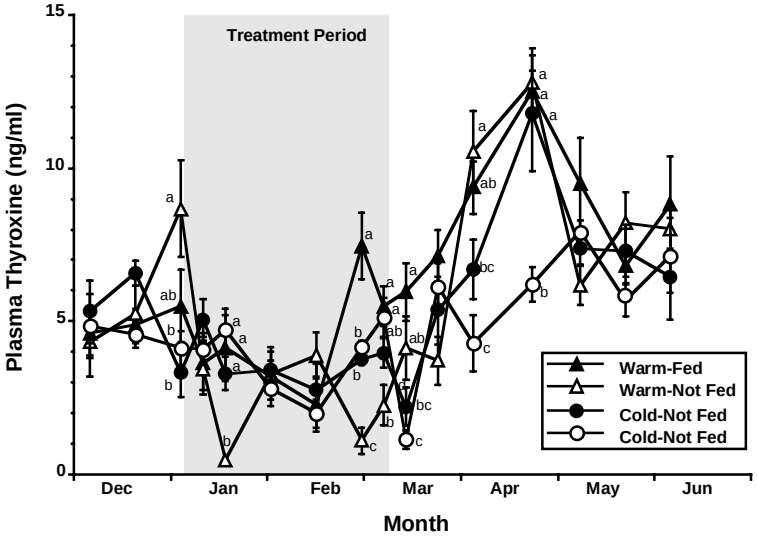

Figure 9. Plasma thyroxine (T4) levels of coho salmon raised under four temperature/feeding regimes. Significant differences among treatments at a given date are indicated by different letters; absence of letters indicates no differences between treatments (n=6 pools of 2 fish/treatment/date).

however, greater fluctuation in the T4 levels of the Warm-Not Fed fish during this period from a transiently high level in early January of approximately 8.0 ng/ml to lows of less than 1.0 ng/ml in both mid-January and late-February. In early March, when all groups were returned to 10ºC and fed a ration of 1.5% (bw/d), T4 levels increased in the Warm-Fed, Warm-Not Fed, and Cold-Fed fish to a peak in late-April of approximately 11.0-14.0 ng/ml before declining in May. In contrast, the T4 levels of the Cold-Not Fed fish increased but only to approximately 7.0 ng/ml by early May. In May there were no significant differences in T4 levels between any of the treatment groups.

DISCUSSION

The aim of this study was to examine the effects of temperature reduction and fasting on juvenile salmon at a developmentally and seasonally appropriate time, i.e., during winter when fish would naturally encounter these conditions. The treatments were based on previous investigations of naturally rearing spring chinook salmon in which there was greater stomach fullness in the summer when temperatures were high and lower stomach fullness in the winter when temperatures were low (Beckman *et al*., 2000). The manipulations of temperature and feeding used did not exactly match what fish rearing in the wild would typically encounter. For example, changes in temperature and feeding would be more gradual and over a longer period than in our study. However, the temperature range tolerated by coho salmon is from 0.5-25ºC with a much narrower optimum rearing temperature ranging from 10-14ºC (Piper *et al.,* 1982). Thus, the temperatures used (2.5-10ºC) fell within the normal physiological range of the animal. A factorial experimental design was employed to isolate the effects of fasting and temperature manipulation on physiological responses. Indices of growth (length and weight), energy storage (body lipid and liver glycogen), and blood levels of three growth and metabolic-regulating hormones (insulin, IGF-I, T4) were determined.

Growth and Temperature

Growth of the fed fish during the treatment was predominantly influenced by temperature. Warm-Fed fish grew steadily throughout the experiment, whereas fish held at low temperature did not grow despite being fed a ration of 0.7% body weight per day. Reduced growth at 2.5ºC would be expected based on bioenergetic considerations (Brett *et al.,* 1969). The capacity for growth of salmon fed to satiation at 2.5ºC is a small fraction of that for fish fed to satiation at 10ºC. The 0.7% ration for the Cold-Fed fish was essentially a maintenance ration since liver glycogen levels and body fat levels were maintained and there was no significant change in body weight or length. However, the 0.7% ration should have provided sufficient protein and energy to allow body growth (Brett et al., 1969). It is possible that the low temperature of the Cold-Fed group suppressed appetite so that the fish did not consume the whole ration, although uneaten food was not seen accumulating in the tanks.

Of the three hormones measured, IGF-I showed the best relationship to body growth. Reduction in temperature from 10ºC to 2.5ºC reduced IGF-I levels in both fed and fasted fish within three days of initiating the change. When the two low temperature groups were returned to 10ºC in March, growth in length and weight resumed within two

weeks for the Cold-Fed group and four weeks for the Cold-Not Fed group. Plasma IGF-I levels in cold treatment groups increased within one week after the increase in temperature in the Cold-Fed group, and within two weeks after in the Cold-Not Fed, which paralleled increases in length and weight. After the treatment period, plasma IGF-I continued to increase to peak levels in April in all groups; growth in length and weight continued at a high and sustained rate from March through May. The effect of temperature on growth and IGF-I levels are in general agreement with those of Beckman *et al.* (1998) who found, in chinook salmon, that fish reared under warmer conditions in the spring had significantly higher plasma IGF-I levels and growth rates than fish reared under relatively cooler water temperatures. Similar results were seen in Atlantic salmon reared at 10º or 2-3ºC (McCormick *et al.*, 2000). The decline in IGF-I when going from warm to cold water is the most rapid change in blood IGF-I levels that we have seen in response to any manipulations, including fasting (discussed below). A rapid increase in IGF-I levels did not occur when temperature was increased. The effect of temperature on IGF-I levels needs further study, especially in relation to temperature effects on IGF binding protein production and binding affinity and IGF-I production and clearance.

Blood levels of insulin did not show a clear relationship to body growth. In contrast to IGF-I, insulin levels increased within three days after the reduction in temperature in both Cold-Fed and Cold-Not Fed groups. After one week of returning the two low temperature groups to 10ºC, plasma insulin levels showed a transient decline and then increased in both groups after two weeks. Thereafter plasma insulin remained near 3-4 ng/ml, similar to levels in December in all groups and similar to the fed groups in January and February. The short term responses of plasma insulin to change in temperature showed interesting patterns; increasing when going from warm to cold and decreasing when going from cold to warm. Changes in blood levels of insulin may be due to changes in secretion and clearance, although we did not measure these parameters in our study. The rapid increase in insulin when fish go from 10º to 2.5ºC may be due to reduced clearance by reduced insulin receptor internalization and turnover in liver and other tissues. In mammalian cells studied in vitro, temperatures lower than 10ºC prevent insulin receptor internalization and turnover (Marshall, 1988). A similar phenomenon has been observed with insulin receptors in hepatocytes of salmonid fish incubated at 15º or 4ºC (Plisetskaya *et al.*, 1993). Although this was not observed in lamprey or frog hepatocytes from 5 to 20ºC (Lappova and Leibush (1995). Nevertheless, changes in hepatic insulin receptor turnover may have a major effect on peripheral plasma insulin levels in fish since the liver removes 30-70% of the insulin to which it is exposed (Plisetskaya and Sullivan, 1989; Carneiro et al., 1993).

The effects of temperature alone on plasma insulin levels in fed fish may involve other mechanisms. In our study plasma samples were collected approximately 16 hours after last feeding in the fed groups, which probably represents basal insulin levels based on studies of brown trout (Navarro *et al.*, 1993) reared at 10-12ºC. However, there are no data on post-prandial changes in plasma insulin in fish held at temperatures as low as 2.5ºC. Thus, an alternate mechanism responsible for the higher transient insulin levels in both the fed and fasted fish at very low temperature may be prolonged retention of food in the gut leading to prolonged nutrient absorption with concomitant nutrient stimulation of insulin release. However, this is only a transient response since the high levels are not maintained in either the Cold-Fed or Cold-Not Fed fish.

As with insulin, plasma T4 levels did not show a clear relationship to body growth. All treatment groups showed a similar pattern in plasma T4 during the study, so there was no consistent response in blood levels of T4 to either temperature or fasting manipulations. During January and February T4 levels were lower than at any other time, and all groups showed increased T4 levels during the spring.

Metabolic Status and Fasting

Fasting fish meet their energy needs primarily by catabolism of lipid and protein; glycogen supplies a small amount of energy, but glycogen is usually the first energy source to be used (Navarro and Gutierrez, 1995). In our study, fasting during the winter reduced liver glycogen content within ten days at both high and low temperature. Glycogen declined to about 70-90% of initial values after two months. In contrast, the Warm-Fed fish maintained high winter liver glycogen levels whereas the Cold-Fed fish showed little change. Others, including Milne *et al.* (1979) and Navarro *et al.* (1993) found liver glycogen to be the most easily mobilized energy reserve in fasted rainbow trout and brown trout, respectively. Similarly, Sheridan and Mommsen (1991) found that liver glycogen levels decreased by 50-60% in coho salmon fasted for 1 and 3 weeks. In contrast with our findings, others have shown that some salmonids conserve liver glycogen stores during fasting (Vijayan *et al.*, 1993) and spawning migration (Mommsen *et al.*, 1980; French *et al.*, 1983) by mobilizing protein and lipid reserves (Phillips *et al.*, 1960; Bilinski and Gardner, 1968; Leatherland and Nuti, 1981; Mommsen *et al.*, 1980; French *et al.*, 1983; Sheridan and Mommsen, 1991).

Whole body lipid levels in fish in the present study were relatively high (7-12%) and seasonally unchanging among all treatments. The two fasted groups had whole body lipid levels that generally ranked lower by approximately 1%, although not significantly lower, than the two fed groups. It was surprising, to observe so little change in whole body lipid levels in association with fasting in this study. Whole body lipid levels measured were quite high compared with levels observed in naturally rearing Yakima River spring chinook (1-8%) (Beckman *et al.*, 2000) but, typical of lipid levels in salmon reared on high-energy dense commercial diets (Shearer *et al.*, 1997; Beckman *et al.*, 1998). Nonetheless, these data demonstrate that in coho salmon that start with very high lipid stores, fasting for as much as two months, even under relatively high winter temperature conditions, results in little depletion of lipid. Hilton (1982) demonstrated in rainbow trout that the prefasting diet might exert substantial influence on fasting metabolism.

Some of the energy needs of the fasted fish in our study may have been met through breakdown of protein, since the two fasted groups, as well as the Cold-Fed group, showed slight decreases (though not statistically significant) in mean body weight in February. It is interesting that the two cold treatment groups had the lowest body weights by March, implying that low temperature may have increased protein breakdown. Glucose production by trout hepatocytes at low temperatures relies more on gluconeogenesis than glycogenolysis (Seibert, 1985). Low temperature has also been shown to enhance gluconeogenesis in red sea bream (Woo and Fung, 1981).

Despite the dramatic declines in liver glycogen, the fasted fish in this investigation demonstrated the ability to recover depleted liver energy stores relatively rapidly (within

1-2 weeks) upon refeeding. Similarly, Farbridge and Leatherland (1992) observed very rapid increases (2-3 days) in HSI in refed rainbow trout following fasting for 6 weeks. Coupled with their rapid ability to recover depleted liver glycogen stores upon refeeding, it would appear that winter fasting, even under warm water conditions, did not compromise the energetic status of the fish in this study. It remains to be determined whether this same conservation of lipid would be possible during different seasons.

Insulin levels were examined in response to fasting because it is one of the key endocrine factors responsible for regulation of metabolic balance in all vertebrates including fish (Murat *et al.*, 1981; Mommsen and Plisetskaya, 1991; Plisetskaya, 1995). It is released in response to nutrition and stimulates glycogen synthesis, lipogenesis, muscle growth and bone growth. In our study, fasting reduced plasma insulin levels within 10 days in fish at 10ºC, but in fish at 2.5ºC it took over one month for a significant difference to develop in plasma insulin in fed and fasted fish. Upon refeeding insulin levels increased to levels equivalent of fed fish within one month. A relatively rapid decline in insulin at moderate temperature corroborate previous studies in cyclostomes and fish that showed insulin levels are depressed during periods of fasting (Murat *et al.*, 1981; Plisetskaya *et al.*, 1976; Plisetskaya *et al.*, 1986; Sheridan and Mommsen, 1991) and elevated during periods of feeding (Tashima and Cahill, 1968; Patent and Foa, 1971; Plisetskaya *et al.*, 1976, 1986; Ince and Thorpe, 1976; Gutierrez *et al.*, 1984; Sower *et al.*, 1985; Sheridan and Mommsen, 1991). The blunted response of insulin to fasting at lower temperature during winter is similar to the findings of Navarro *et al.* (1992) who demonstrated in brown trout that fasting during summer (12-14ºC) caused a more rapid decrease in insulin levels than fasting during winter (8-10ºC). They speculated that this difference might be a result of increased metabolic rate in the summer compared with winter. The higher levels of plasma insulin in Cold-Not Fed fish compared with Warm-Not Fed fish correlate with higher levels of liver glycogen in Cold-Not Fed fish, which suggests that insulin may be protecting liver glycogen stores.

This study examined, for the first time in a teleost, the combined effects of fasting and temperature on plasma IGF-I. For fish at 10ºC, IGF-I levels declined within one month in fasted compared to fed fish. At 2.5ºC, however, it took two months before a difference in IGF-I levels in fed and fasted fish could be detected. The delayed response of IGF-I to fasting in fish at 2.5ºC may be due in part to the general reduction in IGF-I at low temperature. Interestingly, IGF-I levels in fed fish, but not in fasted fish, increased from January to March, probably in response to increasing day length acting through growth hormone secretion. This implies that feeding during winter may be important to maintain sensitivity of the GH-IGF-I axis. In general, IGF-I was slower than insulin in response to fasting and refeeding. Other work has shown that the GH-IGF-I axis is significantly influenced by nutritional status. Fasting has been shown to cause decreases in hepatic production of IGF-I-like activity (Komourdjian and Idler, 1978) and immunoreactive IGF-I-like peptide(s) (Nui *et al.*, 1993) in rainbow trout, and circulating immunoreactive IGF-I levels (Moriyama *et al.*, 1994) and hepatic IGF-I mRNA production (Duan and Plisetskaya, 1993) in coho salmon. Duan and Hirano (1992) showed that fasted eels (*Anguilla japonica*) had significantly lower hepatic IGF-I mRNA and IGF-I bioactivity than fed controls. Silverstein *et al*. (1998) correlated higher rations with elevated plasma IGF-I levels in chinook salmon. Finally, in the gilthead seabream,

Sparus aurata, manipulation of ration and protein content of feed has also been correlated with circulating plasma IGF-I levels (Perez-Sanchez *et al.*, 1994, 1995).

In salmonids, the thyroid hormones, thyroxine (T4) and triiodothyronine (T3) have been shown to play important roles in both metabolism (Plisetskaya *et al.*, 1983) and smoltification (Hoar, 1988; Folmar and Dickhoff, 1980). In fish, much evidence suggests that thyroid activity parallels that of the ambient water temperature (Gorbman, 1969; Eales, 1979). Furthermore, numerous studies in salmonids have shown that long-term fasting (several days to several weeks) decreases both plasma T4 and T3 (see Eales, 1988). In the present investigation, however there was little difference in plasma T4 levels during the winter between warm and cold acclimated fish, which were either fed or fasted. These findings are in general agreement with those of Eales *et al.* (1982) in rainbow trout and (Leloup and DeLuze, 1985) in the eel (*Anguilla anguilla*) who showed that maintenance of fish at high and low temperatures did not significantly affect plasma T4 levels. These investigators did, however, find that temperature change altered T4 production as well as clearance rate, degradation rate, and conversion of T4 to T3. Thus, it would appear that the thyroid axis is in fact highly influenced by temperature, but plasma T4 levels remain relatively constant under varying temperature conditions. The effect of temperature may have been much more profound on plasma T3 levels in this study, however, we did not have sufficient plasma to measure T3. It should be noted that the previously mentioned work reviewed by Eales (1988) was all conducted under a 12L-12D constant photoperiod. Our findings suggest that the seasonal change in photoperiod has a more significant effect on the plasma profiles of T4 than either temperature or ration.

Seasonal endocrine patterns

Most temperate zone fishes show seasonal differences in growth; low growth in the winter and increasing growth in the spring. Seasonal growth is influenced by environmental changes in photoperiod, temperature and food availability, among other factors. A general pattern is emerging of endocrine correlates of seasonal growth in several teleost species, with vernal increases in production and release of GH, IGF-I, and thyroid hormones (Komourdjian *et al.*, 1976; Marchant and Peter, 1986; Bjornsson *et al*., 1989; Perez-Sanchez *et al.*, 1994, 1995; McCormick *et al*., 1995, 2000; Beckman *et al.*, 2000). Seasonal changes in insulin levels have been studied in salmon, and show increases to peak values in late winter or early spring (Plisetskaya *et al.*, 1988; Dickhoff *et al.*, 1989; Gutierrez and Plisetskaya, 1991; Duan *et al.*, 1995). The yearling coho salmon used in this study showed increased IGF-I and thyroid hormones in the spring. For the most part, the winter treatments did not influence the elevation of IGF-I and T4 during spring, except for slightly lower values for IGF-I and T4 in the Cold-Not Fed group. Plasma insulin increased to a peak value in late winter in all groups except the Cold-Not Fed fish. Thus, the winter treatments did not have a major effect on the general increases in these hormones going from winter to spring.

The yearling coho salmon used in this study also underwent the parr-to-smolt transformation (smoltification) during March to May, and the hormones measured have also been identified to play various roles in controlling smoltification. There was no effect of winter temperature or fasting on subsequent smoltification of these fish in spring

(Larsen *et al.*, 2001 or Chapter 3 A above), which agrees with the lack of significant effects of the treatments on the increases in hormone levels during mid- to late spring.

Conclusions

Naturally rearing salmonids inhabit an environment with seasonal fluctuations in both temperature and food availability resulting in very dynamic physiological changes. This study revealed different changes in hormones regulating growth and metabolism in response to ecologically appropriate winter treatments of low temperature and fasting. Low temperature reduced fish growth and caused rapid declines in plasma IGF-I levels, increases in insulin levels and no effect on T4 levels. Fasting depressed plasma insulin more rapidly than plasma IGF-I at 10ºC, but fasting at 2.5ºC had a much reduced effect on plasma levels of both insulin and IGF-I. Fasting reduced liver glycogen, but did not have major effects on body lipid levels. However, it must be emphasized that these results were obtained under a winter photoperiod. During short winter days photoperiodic stimulation of growth hormone production is likely to be low. In future studies similar experimental manipulations should be conducted under fall, spring and summer photoperiods to gain a true understanding of the fasting and temperature effects in seasonally varying environments.

Caution must be exercised in speculating on the significance of changes in blood levels of the three hormones measured in light of the importance of hormone production versus clearance, types and amounts of IGF binding proteins, receptor regulation, autocrine and paracrine production of IGF, and tissue conversion of T4 to T3, among other factors. Nevertheless, with these caveats in mind, it is justified to discuss the potential significance of trends in hormone levels seen in response to temperature and feeding within a seasonal context. Most notably, the present findings of divergent effects of temperature on circulating levels of insulin and IGF-I merit additional study on the mechanisms of temperature effects on hormone production, clearance, receptor turnover, and IGF binding proteins, among others. The physiological significance of temperature effects on insulin family hormones is particularly relevant to understanding hormonal regulation of seasonal growth in poikilotherms.

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